International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF NADIFLOXACIN AND MOMETASONE FUROATE USING HPLC

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***Article History:**

Received: 20/02/2026
Revised: 01/03/2026
Accepted: 28/03/2026

ABSTRACT

A simple, precise, accurate, and robust reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Nadifloxacin (NFC) and Mometasone Furoate (MSF) in pharmaceutical dosage forms. Chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase consisting of 10 mM KH_2PO_4 and methanol in the ratio of 20:80 (v/v), maintained at a flow rate of 1.0 mL/min. Detection was carried out at 254 nm. The retention times for NFC and MSF were found to be approximately 3.77 min and 5.97 min, respectively. The method was validated in accordance with ICH guidelines. Linearity was observed over the concentration range of 1–5 µg/mL for NFC and 2–10 µg/mL for MSF, with correlation coefficients of 0.9985 and 0.9996, respectively. The method demonstrated good accuracy, with percentage recoveries ranging from 98.57% to 99.03% for NFC and 98.77% to 99.28% for MSF. Precision studies showed %RSD values less than 2%, indicating high reproducibility. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.10µg/mL and 0.32µg/mL for NFC, and 0.25 µg/mL and 0.75 µg/mL for MSF, respectively. The developed method was successfully applied for the assay of pharmaceutical formulation, with assay values of 98.00% for NFC and 99.00% for MSF. The proposed method is suitable for routine quality control analysis of Nadifloxacin and Mometasone Furoate in combined dosage forms.

Keywords: Nadifloxacin, Mometasone Furoate, RP-HPLC, Method Development, Method Validation, ICH Guidelines, Linearity, Accuracy, Precision, Pharmaceutical Analysis.

INTRODUCTION

Nadifloxacin and Mometasone Furoate are widely used in combination for the topical treatment of inflammatory bacterial skin infections (Narayanan *et al.*, 2014). Nadifloxacin is a topical fluoroquinolone antibiotic that exhibits broad-spectrum antibacterial activity by inhibiting bacterial DNA gyrase and topoisomerase IV, thereby preventing DNA replication and transcription. It is particularly effective against *Propionibacterium acnes* and *Staphylococcus* species. On the other hand, Mometasone Furoate is a potent synthetic corticosteroid with anti-inflammatory, antipruritic, and vasoconstrictive properties, commonly used to reduce inflammation, erythema, and itching associated with dermatological conditions (Mitscher; 2005).

The combination of Nadifloxacin and Mometasone Furoate provides a synergistic therapeutic effect by simultaneously addressing infection and inflammation, making it highly effective in the management of acne vulgaris and other inflammatory dermatoses (Nenoff; 2006). Due to their combined use in semisolid dosage forms such as creams and gels, accurate and reliable analytical methods are essential for their simultaneous estimation in pharmaceutical formulations (Bora *et al.,* 2014).

High Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques for the quantitative determination of pharmaceutical compounds due to its high sensitivity, specificity, accuracy, and reproducibility. It enables efficient separation of components based on their interaction with stationary and mobile phases under high pressure conditions. Proper optimization of chromatographic parameters such as mobile phase composition, flow rate, column type, and detection wavelength is critical for achieving satisfactory resolution and peak symmetry (Kupiec, 2004).

Several analytical methods including HPLC (Abdallaha *et al.,* 2020), and HPTLC (Zanwar *et al.,* 2020; Zanwar *et al.,* 2024), have been reported for the estimation of Nadifloxacin and Mometasone Furoate either alone or in combination with other drugs. These methods have demonstrated good linearity, precision, and accuracy when validated according to International Conference on Harmonisation (ICH) guidelines. However, there remains a need for simple, rapid, cost-effective, and robust RP-HPLC methods for routine quality control analysis of these drugs in combined dosage forms.

Analytical method validation is an essential process to confirm that the developed method is suitable for its intended purpose. According to ICH guidelines, validation parameters include specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability. A validated method ensures reliability and reproducibility of results, which is crucial for regulatory compliance and quality assurance in pharmaceutical industries.

Therefore, the present study aims to develop and validate a simple, precise, accurate, and robust RP-HPLC method for the simultaneous estimation of Nadifloxacin and Mometasone Furoate in pharmaceutical dosage forms.

MATERIALS AND METHODS

Materials

Nadifloxacin (NFC) and Mometasone Furoate (MSF) were obtained as gift samples from a reputed pharmaceutical company. HPLC grade methanol and analytical grade potassium dihydrogen phosphate (KH_2PO_4) were procured from Merck India Ltd. Orthophosphoric acid used for pH adjustment was of analytical grade. All chemicals and reagents used were of suitable analytical or HPLC grade. Double distilled water was used throughout the study. The marketed cream formulation containing Nadifloxacin and Mometasone Furoate was purchased from a local pharmacy and used for analysis.

Methods

Selection of Mobile Phase

Initially, several mobile phase compositions in different ratios were evaluated for the simultaneous estimation of Nadifloxacin (NFC) and Mometasone Furoate (MSF) in fixed dosage form. The selection was based

on system suitability parameters such as retention time (RT), tailing factor, number of theoretical plates, and height equivalent to a theoretical plate (HETP). Among the various combinations tested, a mixture of 10 mM potassium dihydrogen phosphate (KH_2PO_4) and methanol in the ratio of 20:80 (v/v) was found to be most suitable, providing good resolution, sharp peaks, and acceptable system suitability results. The prepared mobile phase was filtered through a 0.45 µm membrane filter to remove particulate matter and subsequently degassed by sonication. The flow rate was maintained at 1.0 mL/min throughout the analysis (Jain *et al.*, 2019).

Selection of Separation Variables

Chromatographic separation was achieved using a C18 (octadecylsilane) column with a dimension of 250 mm × 4.6 mm and particle size of 5 µm. The mobile phase consisted of 10 mM KH_2PO_4 and methanol in the ratio of 20:80 (v/v), with pH adjusted to approximately 3.0 using orthophosphoric acid. Methanol was used as the diluent.

The analysis was carried out in isocratic mode at a flow rate of 1.0 mL/min, with the column maintained at ambient temperature (25 ± 2°C). The injection volume was 20 µL, and detection was performed at 254 nm using a UV/Visible detector.

The retention times were found to be 3.775 ± 0.2 minutes for Nadifloxacin and 5.985 ± 0.2 minutes for Mometasone Furoate. The resolution between the two peaks was greater than 2.0, indicating effective separation. The tailing factor was less than 2.0, and the number of theoretical plates exceeded 2000, confirming good column efficiency. All system suitability parameters were within acceptable limits.

Preparation of Standard Solutions

Preparation of Stock Solution

Accurately weighed 10 mg of Nadifloxacin and Mometasone Furoate were transferred separately into 10 mL volumetric flasks. About 5 mL of methanol was added as diluent, and the solutions were sonicated for 20 minutes to ensure complete dissolution. The volume was then made up to the mark with methanol to obtain a concentration of 1000 µg/mL (Stock-A).

Preparation of Sub-Stock Solution

From Stock-A, 5 mL of each drug solution was transferred into separate 50 mL volumetric flasks and diluted up to the mark with methanol to obtain a concentration of 100 µg/mL (Stock-B).

Preparation of Working Solutions

Aliquots of 0.1, 0.2, 0.3, 0.4, and 0.5 mL of Stock-B were transferred into separate 10 mL volumetric flasks and diluted up to volume with methanol. This resulted in concentrations of 1, 2, 3, 4, and 5µg/mL for Nadifloxacin. Similarly, appropriate aliquots were diluted to obtain concentrations of 2, 4, 6, 8, and 10µg/mL for Mometasone Furoate.

Linearity and Calibration Curve

Linearity of the method was established by preparing a series of standard solutions in the concentration range of 1–5 µg/mL for Nadifloxacin and 2–10 µg/mL for Mometasone Furoate. All solutions were filtered through a 0.45 µm membrane filter prior to injection. Each concentration was analyzed in triplicate, and chromatograms were recorded.

Calibration curves were constructed by plotting mean peak area against concentration, and regression equations were obtained. The

response factor was calculated by dividing the peak area by the corresponding concentration.

System Suitability Parameters

Prior to analysis, the chromatographic system was equilibrated with the mobile phase at a flow rate of 1.0 mL/min until a stable baseline was achieved. Six replicate injections of standard solutions were performed to evaluate system suitability. Parameters such as retention time, theoretical plates, tailing factor, and resolution were recorded to ensure the adequacy of the system for analysis (Jain *et al.,* 2010).

Validation of Developed Method

The developed method was validated in accordance with standard guidelines for the following parameters (Sharma *et al.,* 2012):

A. Linearity

Linearity was assessed by analyzing five different concentrations within the specified range. Each concentration was injected in triplicate, and the mean peak area was calculated. Calibration curves were plotted, and regression equations along with correlation coefficients were determined.

B. Specificity

Specificity of the method was evaluated to ensure that the analytes were clearly resolved from impurities, degradation products, and excipients present in the formulation (Sharma *et al.,* 2009).

C. Accuracy

Accuracy was determined by recovery studies using the standard addition method. Known amounts of standard drug (80%, 100%, and 120%) were added to pre-analyzed samples, and percentage recovery was calculated (Biswas *et al.,* 2018).

D. Precision

Repeatability

Repeatability was assessed by analyzing five replicate samples at different concentration levels within the linearity range. The concentrations used were 1–5 µg/mL for Nadifloxacin and 2–10 µg/mL for Mometasone Furoate. The results demonstrated the precision of the method under the same operating conditions over a short time interval.

Analysis of Formulation

An accurately weighed quantity of cream formulation equivalent to 10 mg of Nadifloxacin was transferred into a 10 mL volumetric flask containing methanol. The solution was sonicated for 25 minutes to ensure complete extraction of the drug, and the volume was made up to the mark with mobile phase. The resulting solution was filtered through a 0.45 µm membrane filter.

The filtrate was further diluted with methanol to obtain final concentrations of 10 µg/mL for Nadifloxacin and 5 µg/mL for Mometasone Furoate. The prepared sample solution was injected into the HPLC system, and the peak areas were recorded.

The amount of drug present in the formulation was calculated using the calibration curve. The analysis was performed in six replicates to ensure accuracy and reproducibility.

RESULTS AND DISCUSSION

The developed RP-HPLC method was found to be simple, precise, and reliable for the simultaneous estimation of Nadifloxacin (NFC) and Mometasone Furoate (MSF) in pharmaceutical dosage form.

The chromatogram of the blank (Figure 1) showed no interfering peaks at the retention times of the analytes, confirming the

specificity of the method. The chromatogram of the standard mixture (Figure 2) exhibited well-resolved, sharp, and symmetrical peaks for both NFC and MSF with retention times of approximately 3.77 min and 5.97 min, respectively, indicating effective separation under optimized chromatographic conditions.

Linearity studies demonstrated that the method exhibited excellent linear response over the concentration range of 1–5 µg/mL for NFC and 2–10 µg/mL for MSF. The correlation coefficients (r^2) were found to be 0.9985 for NFC and 0.9996 for MSF, as presented in Table 1, indicating strong linear relationships between concentration and peak area. The slope and intercept values further supported the sensitivity and reliability of the method.

System suitability parameters were evaluated to ensure the performance of the chromatographic system. As shown in Table 2, the %RSD values for retention time and peak area were found to be less than 2%, indicating good precision of the system. The number of theoretical plates exceeded 2000, and tailing factors were less than 2 for both drugs, confirming acceptable column efficiency and peak symmetry.

Accuracy of the method was determined by recovery studies at 80%, 100%, and 120% levels. The mean percentage recovery ranged from 98.57% to 99.03% for NFC and 98.77% to 99.28% for MSF (Table 3), demonstrating the accuracy of the method. The low %RSD values (<2%) further confirmed the reproducibility of the recovery results.

Precision of the method was assessed in terms of repeatability, intra-day (day-to-day variation), and inter-analyst variation. As summarized in Table 4, the %RSD values for all precision studies were found to be less than 2%, indicating that the method is precise and reproducible under different conditions. Robustness studies also showed minimal variation in results, confirming the reliability of the method against small deliberate changes in experimental conditions. The sensitivity of the method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ). The LOD values were found to be 0.10 µg/mL for NFC and 0.25 µg/mL for MSF, while the LOQ values were 0.32 µg/mL and 0.75 µg/mL, respectively (Table 5). These results indicate that the method is sufficiently sensitive for routine analysis. The developed method was successfully applied for the assay of tablet formulation. The percentage assay results were found to be 98.00% for NFC and 99.00% for MSF with low %RSD values, as shown in Table 6. These results confirm that the method is suitable for the quantitative determination of both drugs in pharmaceutical dosage forms. The developed RP-HPLC method complies with validation requirements and can be effectively used for routine quality control analysis of Nadifloxacin and Mometasone Furoate in combined dosage forms.

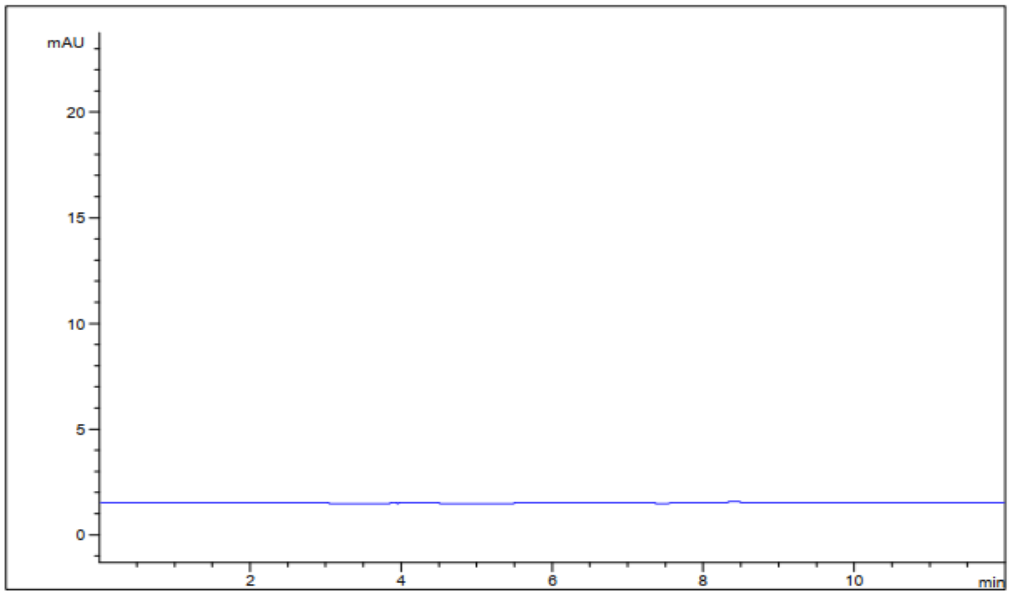

Figure 1: Chromatogram of Blank

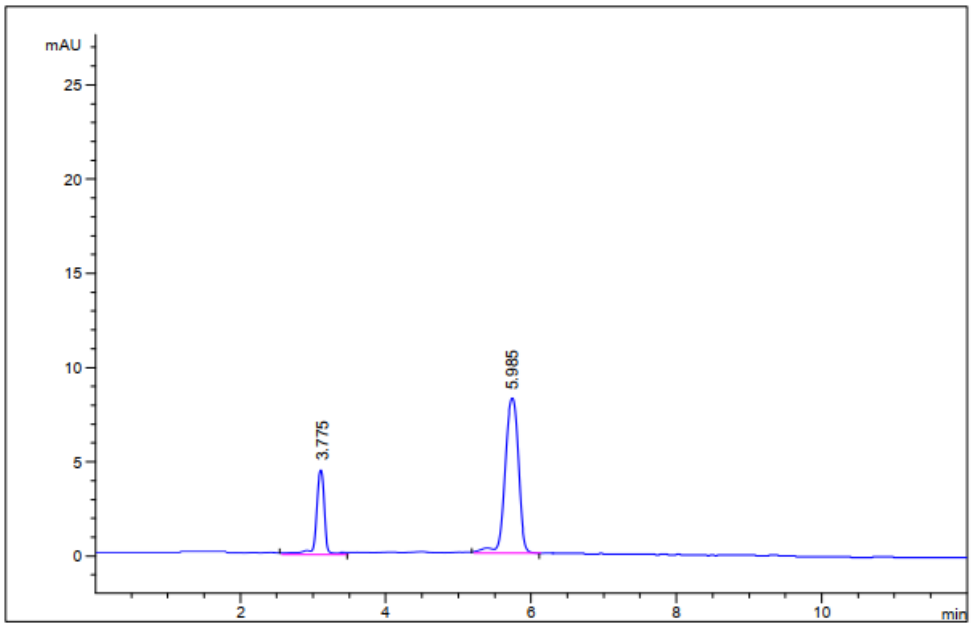

Figure 2: Chromatogram of Both the drug

Table 1: Statistical Summary of Linearity for NFC and MSF

Parameter	Nadifloxacin (NFC)	Mometasone Furoate (MSF)
Linearity Range (µg/mL)	1 – 5	2 – 10
Correlation Coefficient (r^2)	0.9985	0.9996
Slope (m)	984.5	889.21
Intercept (c)	20.689	22.364

Table 2: Statistical Summary of System Suitability Parameters

Parameter	Drug	Mean	Standard Deviation (SD)	%RSD
Retention Time (RT)	NFC	3.775	0.003	0.08
	MSF	5.976	0.007	0.12
Area Under Curve (AUC)	NFC	5015.957	4.732	0.09
	MSF	8945.966	10.858	0.12
Theoretical Plates (N)	NFC	2671.333	14.390	0.54
	MSF	2644.500	49.367	1.87
Tailing Factor	NFC	1.152	0.023	2.00
	MSF	1.157	0.021	1.81

Table 3: Statistical Summary of Recovery Studies

Drug	Level	Mean % Recovery	Standard Deviation (SD)	%RSD
Nadifloxacin (NFC)	80%	98.61	0.481	0.488
	100%	98.57	0.810	0.821
	120%	99.03	0.367	0.371
Mometasone Furoate (MSF)	80%	99.28	0.289	0.291
	100%	98.77	0.824	0.834
	120%	99.16	0.443	0.447

Table 4: Statistical Summary of Precision and Robustness Studies

Parameter	Drug	Mean (%)	SD	%RSD
Repeatability	NFC	98.225	0.036	0.037
Repeatability	MSF	98.903	0.025	0.025
Day-to-Day Variation	NFC	97.930	0.041	0.042
Day-to-Day Variation	MSF	97.920	0.061	0.063
Analyst-to-Analyst Variation	NFC	97.823	0.034	0.035
Analyst-to-Analyst Variation	MSF	98.834	0.047	0.047
Robustness	NFC	98.394	0.028	0.029
Robustness	MSF	97.354	0.097	0.100

Table 5: LOD and LOQ of NFC and MSF

Name	LOD (µg/ml)	LOQ (µg/ml)
NFC	0.10	0.32
MSF	0.25	0.75

Table 6: Result of assay of tablet formulation

	NFC*	MSF*
Label Claim (mg)	**1%**	**0.1%**
% Found (mg)	0.98	0.099
% Assay	98.00	99.00
% RSD	0.125	0.265

*Average of three determination

CONCLUSION

A simple, rapid, precise, and accurate RP-HPLC method was successfully developed and validated for the simultaneous estimation of Nadifloxacin and Mometasone Furoate in pharmaceutical dosage form. The optimized chromatographic conditions provided well-resolved peaks with acceptable retention times and satisfactory system suitability parameters. The method exhibited excellent linearity over the selected concentration ranges with high correlation coefficients. Accuracy studies demonstrated good recovery, while precision studies confirmed the reproducibility of the method with low %RSD values. The method was also found to be robust and sensitive, as indicated by low LOD and LOQ values. The developed method was successfully applied for the assay of marketed formulation, yielding results within acceptable limits. Therefore, the proposed RP-HPLC method is reliable and suitable for routine quality control analysis of Nadifloxacin and Mometasone Furoate in combined pharmaceutical dosage forms.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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