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RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF NAFTIFINE HYDROCHLORIDE IN FORMULATED PRODUCTS: DEVELOPMENT AND VALIDATION

Kajal Sunil Shinde[1*], Chandraprabhu Motichand Jangme[2], Abhinandan Raosaheb Patil[3]

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ABSTRACT
Background: Naftifine hydrochloride is an allylamine antifungal agent commonly used to treat dermatophyte infections. It inhibits squalene epoxidase, a key enzyme in ergosterol biosynthesis, thereby disrupting the integrity of the fungal cell membrane. It exhibits broad-spectrum activity against dermatophytes, yeasts, and molds, and is typically formulated as a 1% topical cream or gel. **Methodology:** A rapid and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of naftifine hydrochloride in a topical cream formulation (2% Naftifast, Zydus), in accordance with ICH and FDA guidelines. Chromatographic separation was achieved on an Inertsil ODS column using an isocratic mobile phase consisting of 35% acetonitrile, 40% methanol, 25% water, and 0.8% triethylamine (pH adjusted to 5.5 with acetic acid) at a flow rate of 1.4 mL/min. Detection was performed at 265 nm. **Results and Discussion:** Naftifine hydrochloride showed a retention time of approximately 4.0 minutes with a total run time of 6.0 minutes. The method displayed excellent linearity over a concentration range of 20–120 µg/mL ($R^2 > 0.999$). Recovery studies indicated a mean recovery of 100.4%. Precision was confirmed by relative standard deviation (RSD) values of less than 2%, demonstrating the method's reproducibility. **Conclusion:** The proposed RP-HPLC method is simple, precise, and time-efficient. It is suitable for routine quality control of naftifine hydrochloride in pharmaceutical dosage forms due to its short analysis time and strong validation performance.

INTRODUCTION

Fungal infections affect millions of individuals globally each year and pose a significant threat to public health, particularly in immunocompromised populations. These infections may range from superficial skin conditions to severe systemic diseases. Among the available antifungal agents, naftifine hydrochloride,

[1]*Dept. of Pharmaceutical Science, Centre for Interdisciplinary Research, D. Y. Patil Education Society (Deemed to be University), Kolhapur, 416006, Maharashtra, India.*
[2]*Department of Pharmacology, D. Y. Patil College of Pharmacy, D. Y. Patil Education Society, Deemed to Be University, Kolhapur 416006, Maharashtra, India.*
[3]*Department of Pharmaceutics, D. Y. Patil College of Pharmacy, D. Y. Patil Education Society, Deemed to Be University, Kolhapur 416006, Maharashtra, India.*

For Correspondence:** **kajalshinde893@gmail.com

a synthetic allylamine derivative, has demonstrated high efficacy against a broad range of fungi, including dermatophytes, yeasts, and molds. Its antifungal activity results from the inhibition of squalene epoxidase, a key enzyme in the ergosterol biosynthesis pathway, leading to disruption of the fungal cell membrane's integrity and ultimately, cell death. Clinically, naftifine is commonly used in topical formulations to treat dermatological infections such as tinea corporis (ringworm), tinea cruris (jock itch), and tinea pedis (athlete's foot) [1–5]. Critical information on linearity, accuracy, precision, detection limits, and quantitation constraints is provided, making it a critical step in developing new dosage forms. The goal of validating an analytical technique, according to the ICH criteria, is to prove that it is suitable for its intended usage. Regulatory agencies increasingly need validation data to be submitted during the creation of medications. Validation guidelines are provided by both the USP and the ICH [6 - 9]. Recent literature has reported the use of HPLC methods employing small-volume phenyl columns and gradient elution for simultaneous quantification of naftifine and its degradation products, achieving very short retention times and reduced solvent usage. While effective for impurity profiling, such methods often require specialized columns and instrumentation. In contrast, the present study offers a simpler, cost-effective approach using isocratic elution with a standard ODS column, enabling rapid (~4 min) and accurate quantification, suitable for routine quality control of cream formulations [10].

MATERIALS AND METHODS

Instrumentation: To create and verify the liquid chromatography method, an isocratic reverse-phase (RP) HPLC approach was used. A column made of Inertsil ODS, with dimensions of 150 mm × 4.6 mm, was used by Scientific. Chemstation software (HPLC AGILENT MODEL 1100) was utilized to process and analyze the data [10].

Chemicals and Reagents: Naftifine hydrochloride (figure 1) procured from Baoji Guokang Bio-Technology Co., Ltd. China Dodal Enterprise Pvt. Ltd. supplied all of the chemicals, which were of HPLC quality. It was SD Fine Chem Limited from whom the triethylamine was bought.

Chromatographic condition

A 150 mm × 4.6 mm, 5 µm Inertsil ODS column was used at a temperature that is actively maintained at 30 ± 0.5°C using a column oven to ensure consistent chromatographic performance. The mobile phase used in the chromatography system consisted of 35% acetonitrile, 40% methanol, 25% water, and 0.8% triethylamine, with the pH adjusted to the target value using acetic acid. This mixture was used to achieve the separation. Quantification was carried out at a 265 nm wavelength, with the mobile phase being pushed in column flow rate (1.4 mL/min). The mobile phase was first filtered using 0.45 µm nylon millipore membranes under vacuum. It was then degassed by ultrasonication for 20 minutes before use. The volume injected was 20 µL, and the injection rate was 1.4 mL/min. The mobile phase composition—acetonitrile, methanol, water, and triethylamine was optimized through several trial runs to achieve sharp peak symmetry, minimal tailing, and short retention time. The use of 0.8% triethylamine at pH 5.5 helped in peak sharpening by minimizing peak tailing due to interaction with residual silanol groups on the stationary phase. A detection wavelength of 265 nm was selected based on the UV absorbance maxima (λmax) of naftifine hydrochloride, ensuring optimal sensitivity and signal-to-noise ratio. Under the optimized chromatographic conditions, naftifine hydrochloride exhibited a retention time of approximately 4.0 minutes, ensuring efficient & rapid elution [11-12].

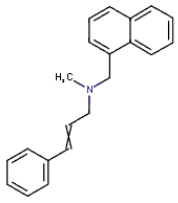


Figure 1: Chemical structure of naftifine

Preparation of Standard Solution

Accurately weigh 100 mg of Naftifine hydrochloride and transfer it to a 100 mL volumetric flask. Add approximately 50 mL of mobile phase to dissolve the drug completely, and then bring the volume up to 100 mL with mobile phase to obtain a stock solution of 1 mg/mL.

Preparation of Sample Solution

Accurately weigh 5 g of the commercial topical cream (2% Naftifast, Zydus) and transfer it to a 100 mL volumetric flask. Add 70 mL of mobile phase and warm the mixture in a water bath at 70°C for 10 minutes with intermittent shaking. Once cooled, it was reconstituted with the mobile phase. Following filtration through a PTFE filter, 5 mL of the filtrate was added to a 50 mL volumetric flask by pipetting it with mobile phase.

Validation Method: This method adhered to ICH and FDA regulations [13–14].

Specificity: A defining feature of HPLC is its ability to differentiate between the analyte and the constituents of the complex mixture. The specificity of the method was assessed by injecting 20 µL volumes of placebo, sample, standard & blank [15 - 16].

Linearity: The linearity and range of the method were evaluated by preparing a series of naftifine hydrochloride solutions (20, 40, 60, 80, 100, and 120 µg/mL), with triplicate injections (n =3) at each concentration level to ensure precision through dilution of the standard stock solution with the mobile phase. Each concentration level was injected in triplicate under identical chromatographic conditions to ensure accuracy and reproducibility. [17].

Sensitivity: The LOD and LOQ were found by analyzing several Naftifine hydrochloride solutions. LOQ is a concentration that produces a signal-to-noise ratio of about 10:1 by RSD (n=3) of > 10%. In contrast, the alternative definition of LOD is a concentration that produces a signal-to-noise ratio of around 3:1 [18].

Accuracy: The accuracy of the assay technique was evaluated by injecting triplicate samples of each concentration and conducting recovery studies at 50%, 100%, and 150% recovery rates. The percent recovery and relative standard deviation (RSD) were calculated for the added amounts of naftifine to evaluate method accuracy [18 – 19].

Robustness: It was established by making minor, intended adjustments to experimental limits, like: Flow rate: ±0.2 mL/min, Column temperature: ±5°C, Composition of mobile phase, organic content: ±5%, Wavelength: ±3 nm
Deliberate variations in method parameters were introduced to assess the procedure's robustness. To evaluate the data for every case, the % recovery and % RSD were measured [18, 20].

Precision: To determine the system's accuracy, ten observations were made on the same day using a standard solution at 100% concentration. Six separate assays using the sample solution at 100% concentration levels demonstrated the accuracy of the procedure on the same day. The RSD values were used as a measure of method repeatability [18 – 19].

RESULTS

Numerous physicochemical assets of naftifine have been culled from published sources. An analytical method was developed to systematically optimize key RP-HPLC chromatographic parameters, including the sample preparation protocol, mobile phase composition, stationary phase selection, and detection wavelength. Several optimization trials were conducted, including modifications in chromatographic conditions and the ratio of acetonitrile to methanol. Isocratic RP HPLC column using a size of 150 cm × 4.6 mm & a particle size of 5 µm.

Method Validation

Specificity

To determine specificity, the chromatograms of the sample solution, the standard solution, and the blank mobile phase were compared. To achieve this, 20 µL of every solution was introduced separately into the system. The results of the chromatogram are presented in Figures 2-4. Specificity was confirmed by the deficiency of coeluting peaks at the retention time of Naftifine hydrochloride interference, indicating the purity of an analyte peak [21]. Although diode array detection (DAD) was used for chromatographic analysis, peak purity data were not captured or archived during the method development process. Therefore, spectral confirmation of peak homogeneity could not be included. Nevertheless, the absence of interfering peaks in blank and placebo chromatograms supports the specificity of the method. The sample preparation procedure was validated for reproducibility, as demonstrated by consistent results across replicate extractions & assays. Additionally, matrix interference was evaluated through a specificity study using blank & placebo cream formulations, which showed no interfering peaks at the retention time of naftifine hydrochloride.

Linearity and Range

An analytical technique is considered linear if, within a specific range, it consistently yields linear test results. By graphing the average HPLC peak area against the correct concentration, the calibration graph was generated. A range of 20-120 µg/mL was used to demonstrate linearity for Naftifine hydrochloride, as shown in Figure 5. The analyte conc. & the area under the peak exhibited a linear relationship, which was confirmed through regression analysis. The regression equation for naftifine HCl was found to be $y = 45.58x – 5.507$, with a correlation coefficient (r^2) of 0.999, indicating excellent linearity. To enhance the robustness of the linearity assessment, the standard error of the slope & intercept were calculated & found to be 0.462 and 36.00,

respectively. These values support the statistical reliability of the linear regression model. Although residual plots were not included in this study, the minimal variation in replicate responses & high correlation coefficient affirm the linear relationship between concentration and response.

LOD & LOQ

Both LOQ and LOD of an analyte were defined by the lowest concentrations at which the analyte can be consistently detected and quantified, respectively. The limits of detection (LOD) and quantification (LOQ) of naftifine hydrochloride were found to be 5.02 µg/mL and 16.72 µg/mL, respectively [23].

Accuracy

Accuracy was evaluated using triplicate injections (n = 3) at three concentration levels: 50%, 100%, and 150% of the target concentration. The percentage recovery at each level ranged from 97.9% to 102.9%, with %RSD values below 2.0%, indicating good agreement between observed and actual values. As per ICH Q2 (R1) guidelines, the acceptance criterion for recovery in assay methods typically ranges from 98% to 102% for active pharmaceutical ingredients in finished products. The results obtained were within or very close to this acceptable range, confirming the accuracy of the proposed method [24].

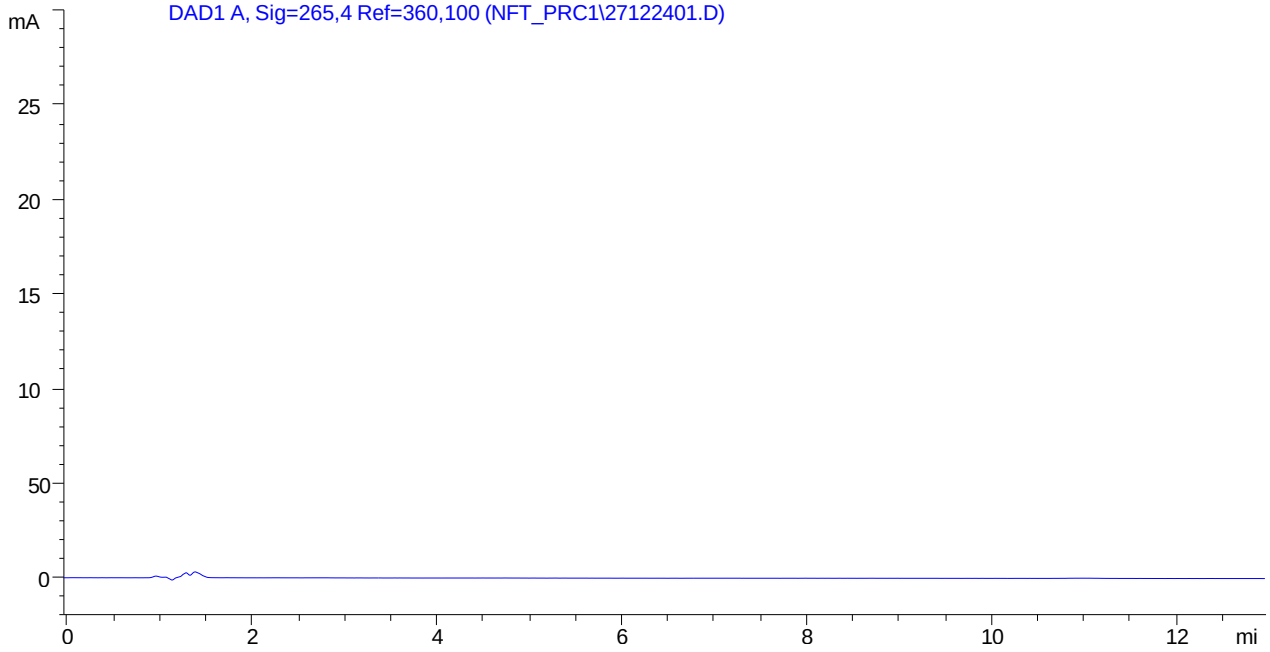


Figure 2: Blank solution chromatogram.

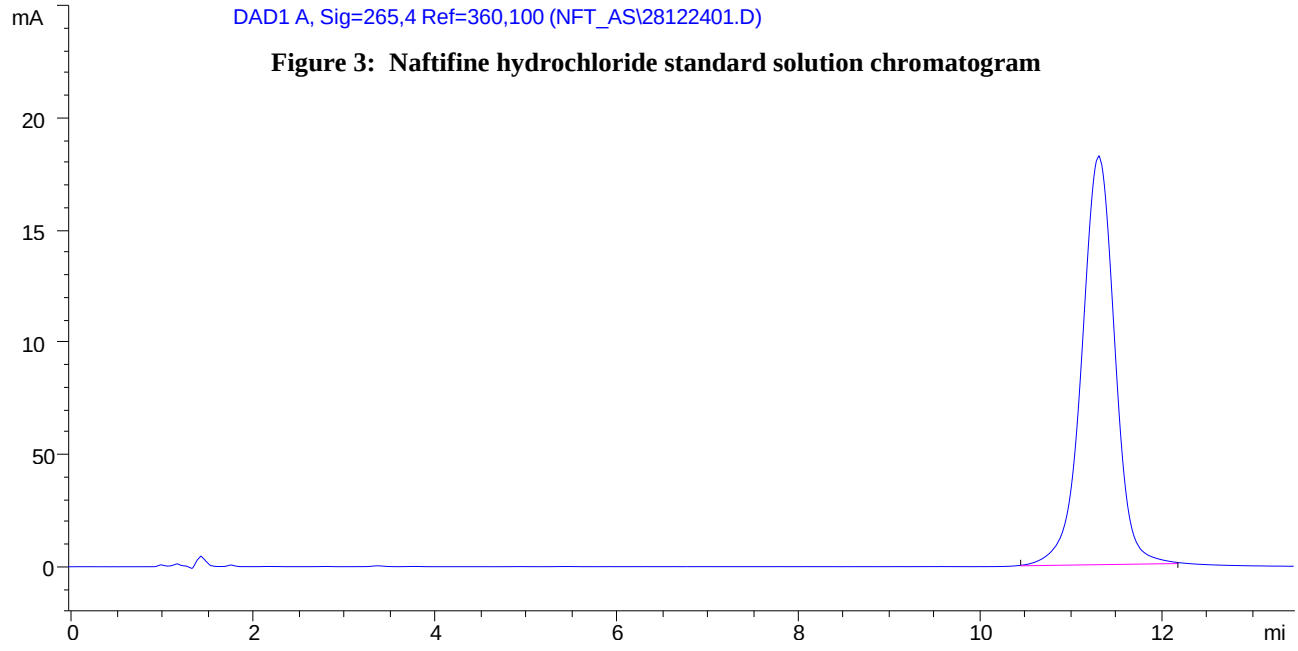


Figure 3: Naftifine hydrochloride standard solution chromatogram

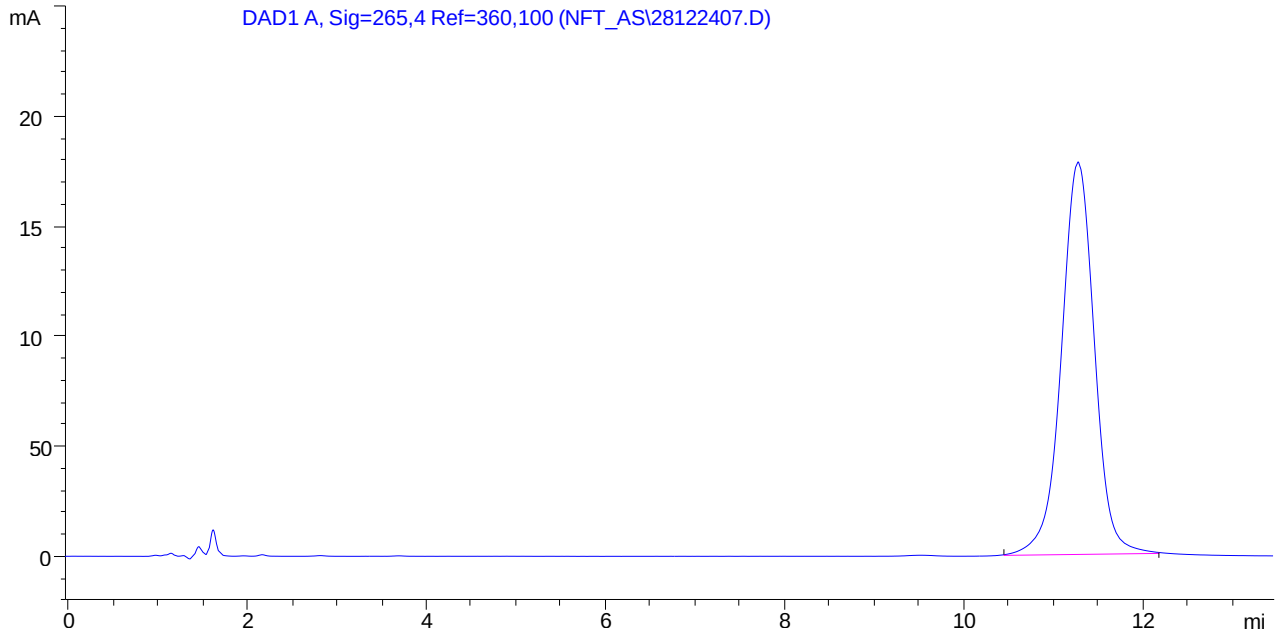


Figure 4: Naftifine hydrochloride sample solution chromatogram

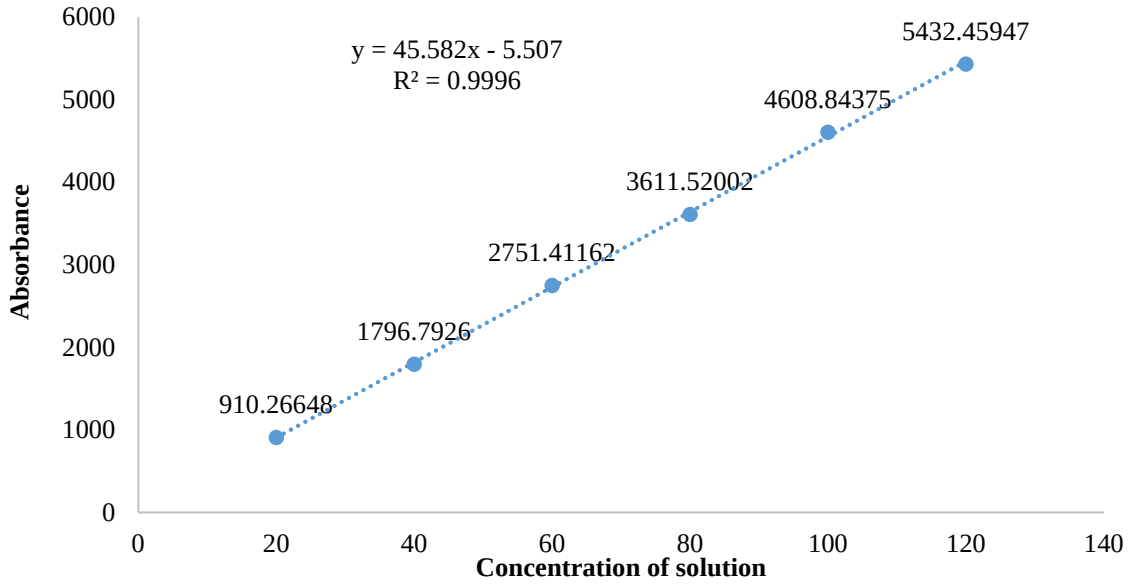


Figure 5: Naftifine hydrochloride standard calibration curve

Precision

The relative standard deviation was used to evaluate the precision of the analytical technique, which is the degree to which a set of measurements from different samplings of the same homogeneous sample, taken under specified conditions, agree with one another. Precision was assessed by evaluating both system and method precision using six replicate injections. The results demonstrated %RSD values below 2.0%, confirming repeatability. The acceptance criteria were defined in accordance with ICH Q2 (R1) guidelines and commonly accepted analytical standards. Specifically, acceptable performance was established as:

- %RSD ≤ 2.0%
- Theoretical plate count > 1000, indicating adequate column efficiency
- Trailing factor ≤ 2.0, ensuring peak symmetry

All values obtained met these criteria, validating the precision of the developed method [25].

Robustness

To support the robustness evaluation, representative chromatograms were included to demonstrate the method's stability under small but deliberate changes in method parameters Figure 6. In all cases, the naftifine HCl peak

remained well-resolved, with no significant shift in retention time, peak shape, or resolution. These findings confirm that the developed method is robust and suitable for routine analysis, even when minor operational variations occur.

Solution Stability

Solution stability was assessed over 24 hours at room temperature and under refrigerated conditions. The standard and sample solutions maintained recovery values within 98.0% to 102.0%, and the %RSD remained below 2.0%, confirming their stability within this timeframe. Although the current study did not extend beyond 24 hours, this duration is considered sufficient for routine analytical use in quality control laboratories where standard and sample solutions are typically used within a single working day. Future studies are planned to evaluate extended stability (e.g., 48–72 hours) to further support long-term use in high-throughput settings.

Table 1: HPLC method recovery data

% of spiked level	Replicate number	Peak area	% of recovery	Mean % RSD
50	1	2863.203	102.9	101.1
	2	2717.328	100.5	
	3	2725.190	99.8	1.6
100	1	5304.486	100.1	100.3
	2	5297.414	99.9	
	3	5294.146	97.9	1.2
150	1	8152.877	101.5	98.7
	2	8147.336	101.4	
	3	8145.226	100.4	0.6
% recovery Mean	98.0–102.0		100.4	
% RSD	Max 2.00		1.13	

n = 3; Acceptance criteria: % Recovery within 98–102% as per ICH Q2 (R1).

Table 2: System precision statistics designed for the standard solution in the projected HPLC technique

Number of Replicates	Theoretical plates Number	RT	Peak area	Tailing factor
1	1.914	11.306	4614.17725	1.02
2	1.925	11.308	4609.46240	1.03
3	1.914	11.308	4614.17725	1.02
4	1.964	11.305	4614.53271	1.02
5	2.003	11.303	4624.70117	1.01
6	2.003	11.308	4611.48584	1.01
Average **%RSD**	1.954 2.16%	11.306	-	1.01 0.74%

n = 6; Acceptance criteria: %RSD ≤ 2.0%, Tailing factor ≤ 2.0, and Theoretical plates >1000.

Table 3: Method precision analysis of the sample solution using the projected HPLC method

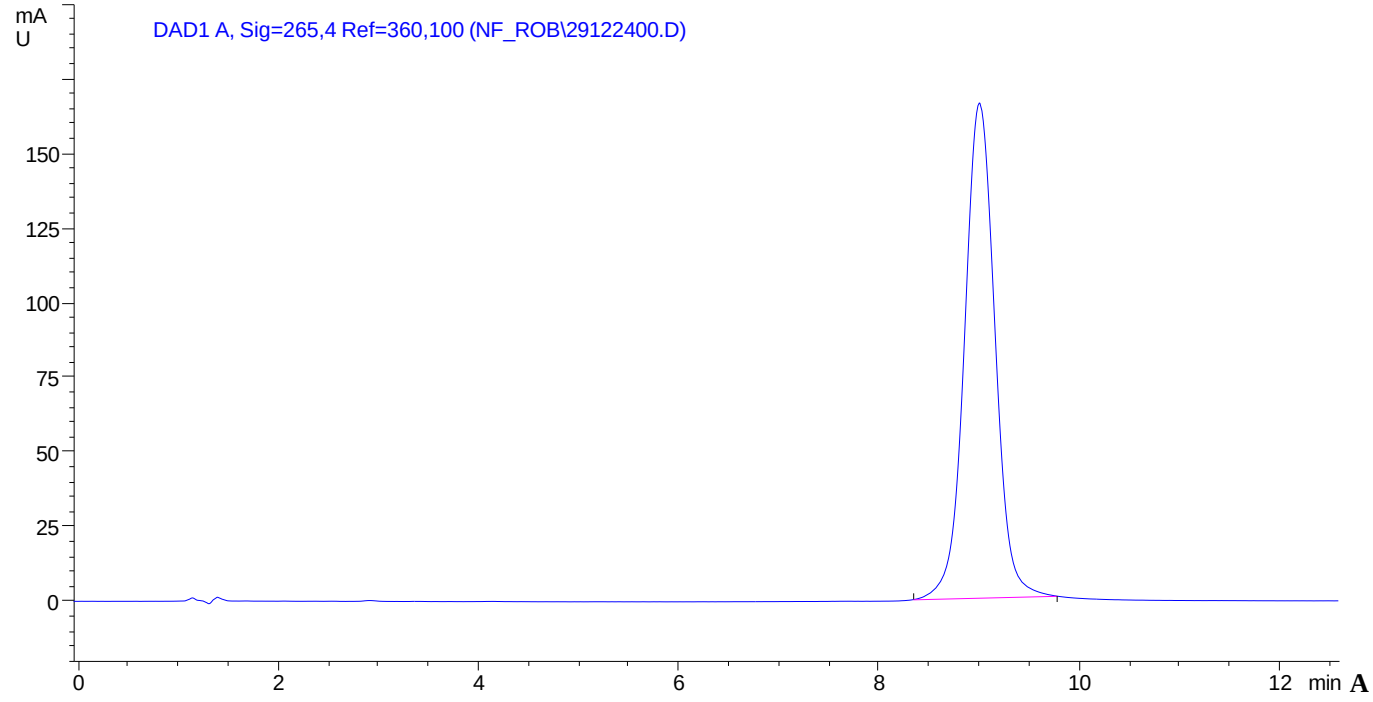
Number of Replicates	RT	Peak area	Theoretical plates Number	Tailing factor
1	11.275	2751.41162	1.914	1.03
2	11.276	3611.52002	1.914	1.03
3	11.285	4608.84375	1.918	1.03
4	11.288	5432.45947	1.957	1.02
5	11.257	910.26648	1.907	1.03
6	11.271	1796.79260	1.876	1.04
Average **%RSD**	11.275	-	1.9143 1.36%	1.03 0.61%

n = 6; Acceptance criteria: %RSD ≤ 2.0%, Tailing factor ≤ 2.0, and Theoretical plates >1000.

Table 4: Projected HPLC technique robustness data.

Parameter		%RSD of assay	%RSD of std peak area
Wavelength	265 nm	0.18	0.02
Column temperature	30 °C	0.15	0.03
Mobile phase composition	Acetonitrile 35%: Methanol 40%	0.16	0.05
Flow rate	1.4 mL/min	0.11	0.06

n = 3; Acceptance criteria: %RSD ≤ 2.0% across all tested parameters.



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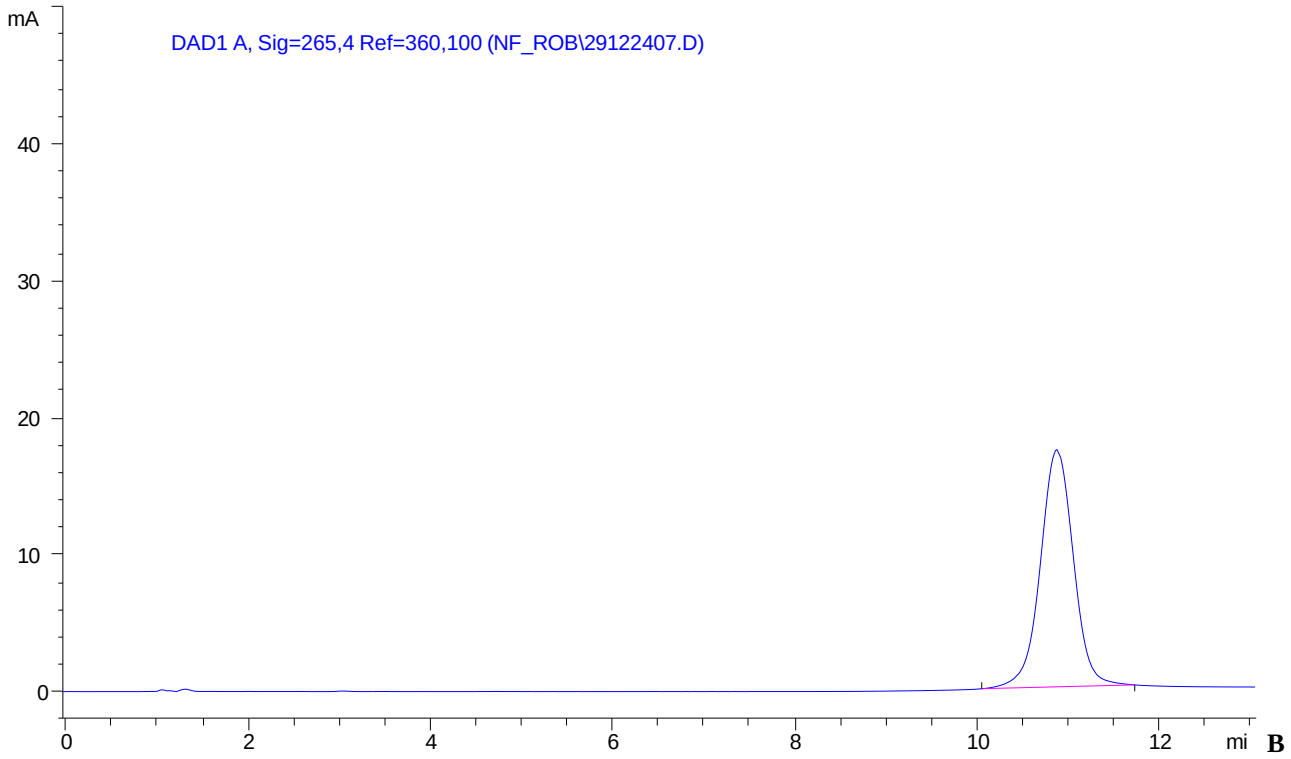

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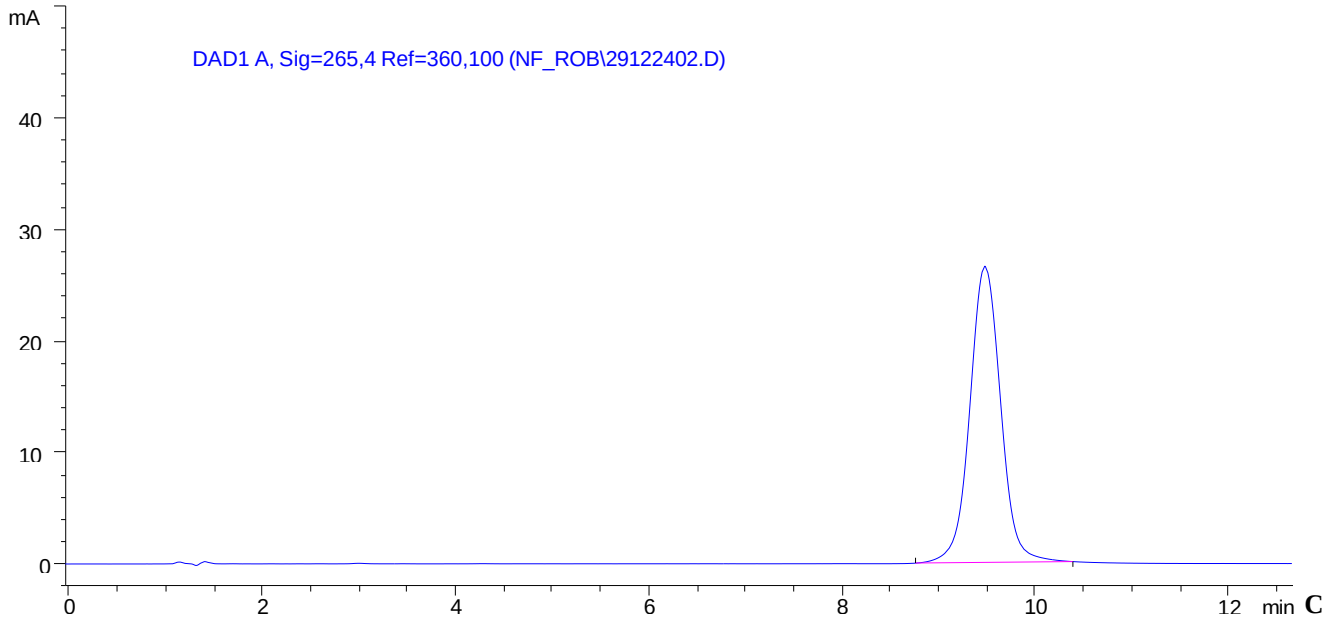

Figure 6: Robustness chromatographs. A shows the standard chromatogram under optimized conditions, B depicts the chromatogram obtained with an increased flow rate (1.5 mL/min), and C presents results with elevated column temperature (35 °C).

DISCUSSION

The developed RP-HPLC method demonstrates high precision, accuracy, and reliability for the quantitative estimation of naftifine hydrochloride in pharmaceutical formulations. The chromatographic conditions were optimized to achieve a retention time of approximately 4 minutes, resulting in a total run time of 6 minutes, which significantly enhances the method's suitability for routine quality control operations. Linearity was observed across a broad concentration range (20–120 µg/mL), with a correlation coefficient (r^2) greater than 0.999, indicating strong analytical performance. Accuracy was confirmed through recovery studies, with percentage recoveries consistently close to 100%. Precision studies yielded relative standard deviation (RSD) values below 2.0%, supporting the method's reproducibility. The method exhibited satisfactory sensitivity, with LOD and LOQ values of 5.02 µg/mL and 16.72 µg/mL, respectively, enabling the detection and quantification of even low levels of naftifine hydrochloride. Robustness studies showed that deliberate variations in analytical parameters—such as flow rate, column temperature, and mobile phase composition—had minimal effect on peak characteristics, confirming the method's reliability under varied conditions. Solution stability studies demonstrated that both standard and sample solutions remained stable for at least 24 hours under ambient and refrigerated conditions, supporting the method's practical application in day-to-day pharmaceutical analysis.

When compared to previously published RP-HPLC methods, the present approach offers several distinct advantages. Many existing methods involve gradient elution or specialized stationary phases, which can increase both analysis time and solvent consumption. In contrast, the current method utilizes a simple isocratic elution system with a conventional C18 column and employs economical and widely available solvents such as methanol and acetonitrile. These features contribute to a more cost-effective and time-efficient workflow, without compromising sensitivity or analytical quality.

Despite its strengths, a key limitation of this study is the absence of forced degradation experiments, which are essential to establish the method as truly stability-indicating. Additionally, the technique has yet to be evaluated for its performance in complex matrices beyond the tested topical cream formulation. Future studies will focus on these aspects, including degradation profiling under stress conditions and the applicability of this approach across a broader range of pharmaceutical dosage forms.

CONCLUSION

A robust, rapid, and reliable RP-HPLC method for quantifying naftifine hydrochloride was successfully developed and validated in accordance with ICH guidelines. The method exhibited excellent resolution and a notably short retention time

of approximately 4 minutes, making it highly efficient for routine quality control purposes. Validation parameters confirmed the method's precision, accuracy, and reproducibility under varied chromatographic conditions, underscoring its applicability in pharmaceutical analysis. Although the method demonstrates strong analytical performance, it does not currently qualify as stability-indicating due to the absence of forced degradation studies. Future research will focus on incorporating such studies to evaluate the method's ability to distinguish between the active drug and its degradation products. In the current context of increasing prevalence of fungal infections and the growing demand for high-throughput analytical techniques, this method offers a timely and valuable contribution to the quality assurance of antifungal therapeutics.

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FINANCIAL ASSISTANCE
NIL

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTION
Kajal Sunil Shinde was responsible for the study design, execution of laboratory experiments, data interpretation, and drafting the initial manuscript. Chandrapraphu Motichand Jangme provided guidance on research methodology, supervised the overall project, and offered substantial input during manuscript revisions. Abhinandan Raosaheb Patil contributed to formulation development and method validation, and reviewed the technical aspects for consistency and accuracy. All authors reviewed and approved the final manuscript and consented to its submission.

ABBREVIATIONS
LOQ – Limit of quantification; RSD – Relative standard deviation; LOD - Limit of Detection; HPLC – High pressure liquid chromatography

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