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A VALIDATED STABILITY-INDICATING HPTLC-PDA METHOD FOR TOLNAFTATE BULK AND STANDARD DRUG

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ABSTRACT: To estimate Tolnaftate in the presence of its forced declination products, a straightforward and quick stability indicating High Performance Thin Liquid Chromatography -Photo Diode Array (HPTLC-PDA) approach was created and validated. The methodology used Toulene: Chloroform: Methanol (311 v/v) as mobile phase and SunQSil C18 column (250mm×4.6mm, 5µm) as stationary phase. Retention time of tolinaftate was found to be 0.27 minutes. To assess the declination conduct of tolinaftate, examinations into acid, alkali, oxidative, UV declination and thermal declination were conducted. In agreement with the criteria of International Conferences on Adjustment (ICH), the created and optimized approach was validated. The results showed that the limits of quantitation and discovery were 0.421 and 0.088µg/mL, singly. With R²=0.9991, linearity was seen in the attention range of 0.276-6µg/mL. Results showed the recovery ranged from 98.2 to 101.01. Reversed 7 Phase -High Performance Thin Liquid Chromatography (RP-HPTLC), a developed and tested technology was used to quantify tolinaftate in an in-house topical result. The main products of Oxidative and Base declination were discovered and described using electrospray ionization mass spectrometry and liquid chromatography.

INTRODUCTION: Tolnaftate is a common treatment for superficial fungal infections, particularly dermatophytoses like ringworm, athlete's foot, and tinea cruris, which falls under the class of Thiocarbamate class¹. It has low systemic absorption and limited water solubility so it's been used typically as creams, sprays, powders and solution forms. It functions by inhibiting the squalene epoxidase enzyme, which is required for synthesis of ergosterol, a vital component of fungal cell membranes². Fungal cell death results from tolinaftate's breakdown of ergosterol production, which weakens membrane integrity and function³.

There is an evident absence of thorough analytical and stability-indicating examinations implementing relevant and advanced chromatographic techniques in the scientific literature, although the drug's decades of clinal consumption, broad antifungal range, and generally moderate toxicity⁴. Since, pharmaceutical quality assurance and regulatory compliance especially that come under the ICH standards are turning into even more crucial and it is essential to develop more precise, validated methods of analysis so that we can identify tolinaftate and monitor its stability under a variety of stress conditions⁵.

The aim of this work is to bridge the gap by developing and validating a stability indicator for measurement of Tolnaftate using HPTLC technique with PDA detector. HPTLC is a powerful chromatographic technique that is an improved version of traditional thin layer chromatography (TLC)⁶.

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It provides excellent resolution, reproducibility, and speedier analysis to handle several samples simultaneously. Ideal for routine analysis and with help of Photo Diode Array (PDA) detector it yields significant analytical benefits. Because it allows for multi-wavelength detection of trace pollutants and offers essential tests. It's a method that helps differentiate the active pharmaceutical in from its breakdown products under stress conditions like acidic, oxidative, thermal and photolytic environments. As per ICHQ1A (R2) recommendation these tests are required to ensure long term safety, effectiveness and shelf life of pharmaceutical products ^{7,8}.

The development of similar methods is necessary since a drug's degradation behaviour reveals details about its formulation issues and packaging needs. The limited number of verified HPTLC-PDA techniques for tolinaftate that are currently published, especially those capable of separating the medication from its possible disintegrate products, makes this project necessary.

Despite there being a few HPLC and UV techniques in the research, they are either not ICH-validated or do not prioritize stability-indicating characteristics. Furthermore, despite their shown efficacy and price ⁹. HPTLC techniques are not widely used in this field. In this context, the current study was conducted to investigate and improve an HPTLC techniques are not widely used in this field. In this context, the current study was conducted to investigate and improve an HPTLC technique when combined with PDA detection that might be used as a stability-indicating test for tolinaftate.

In order to model potential breakdown routes during production, storage, or use, the process involves forcing Tolinaftate to degrade under acid, base, oxidation, heat, and photolytic conditions. Following ICH Q2(R1) suggestions, the devised technique was then verified in terms of linearity, accuracy, precision, robustness, specificity, limit of detection (LOD), and limit of quantification (LOQ), among other aspects ¹⁰. This study's goal was to create a straightforward, accurate, precise, and reproducible HPTLC-PDA approach that could measure tolinaftate and successfully separate it from its breakdown products, acting as a stability

indication ¹¹. The goal was to help the quality assurance of formulations based on tolinaftate by offering an analytical approach that has been confirmed and complies with current industry and regulatory requirements ¹². In addition, because of its operational and economic advantages, the research, aimed to develop a technique that could be regularly applied in quality control labs. Tolinaftate's chemical structure may be depicted in **Fig. 1**.

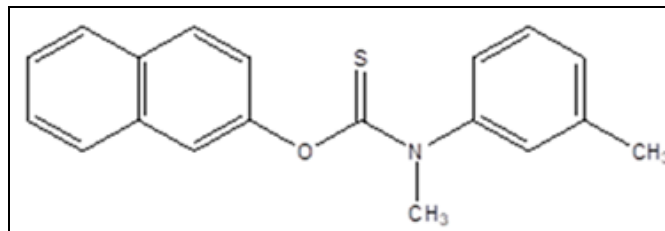


FIG. 1: STRUCTURE OF TOLINAFTATE

In conclusion, the research presented in highly relevant to the pharmaceutical industry today, when regulatory compliance, impurity profiling, and analytical method validation are becoming more and more important ¹³.

This study's validated HPTLC-PDA approach fills an important analytical gap by offering a quick, easy, and economical way to track tolinaftate in standard form and future studies, including formulation development, bioanalytical evaluations, or growth to complicated dosage forms, may be based on this approach, which is anticipated to make a substantial contribution to the regular quality control of formulations containing tolinaftate ¹⁴. By ensuring the supply of stable and effective antifungal medications, the project's outcomes might strengthen regulatory paperwork, refine formulation techniques, and ultimately improve patient safety ¹⁵.

MATERIALS AND METHODS:

Experimental:

Chemicals and Reagents: We bought the standard drug tolinaftate (unadulterated 98 w/ w) from Sigma Aldrich. In Mumbai, India, Fisher Scientific India Pvt. Ltd. supplied the water and HPLC- grade acetonitrile. Modern diligence, Nashik, India, supplied the hydrochloric acid (HCl) Analytical Reagent (AR) grade and sodium hydroxide (NaOH) Extra Pure (EP) grade.

Instrumentation HPTLC Analysis: The JASCO (Japan) HPTLC system 4000 series, which includes a force stage (BS-4000-1), quaternary pump (PU-4180-LPG), degasser, autosampler (AS4050), and photodiode array sensor (MD-4017), was used for the HPLC tests and between system factors and particular computer the LC-Net 2/ADC was served as a tackle interface. The program ChromNAV interpretation 2.0 was used to examine each of the chromatograms that were acquired.

Preparation of in-house Expression: A 100 ml beaker was filled with 100 mg of medication, which had been carefully counted. A sonicator was used to mix it after 5.5 ml of isopropyl alcohol was introduced and gradually methanol was added. A medicine result with a 1 w/v attention was also acquired by mixing the result with 5.5 mL of isopropyl myristate. Using Whatman sludge paper No. 1, the attendant result was filtered and also stored in the proper vessel until it was required.

Preparation of Standard Result: Ten milligrams of tolinaftate were dissolved in one hundred milliliters of mobile phase to produce a standard stock result that contained 100 µg/mL of tolinaftate. After being sonicated, the result was filtered using an Axiva 0.2- µ sample purifier.

Preparation of Sample Result: A standard tolinaftate result (10,000 µg/mL) was adulterated by transferring 1 mL into a 10 mL volumetric beaker, followed by sonication with the mobile phase for 15 minutes to gain a 1,000 µg/mL working result. From this, 0.1 mL was further adulterated to 10 mL to yield a 10 µg/mL test result. The result was assayed in triplet using HPTLC, and the tolinaftate content in the in-house topical expression was calculated grounded on the results, the result was filtered using an Axiva 0.2- µ sample sludge.

Chromatographic Conditions: The needed issues were attained when acetonitrile and water were combined in an 85/15, v/v rate. Using Toulene: Chloroform: Methanol (311, v/v) as the eluent, tolinaftate was separated from its declination product on a SunQSil C-18 (250 mm × 4.6 mm, 5 µm) column. The eluent inflow rate was 1 milliliter per minute. One run of analysis took ten minutes to perform. A finding was carried at 255 nm and 20 µl

of material was extracted and added in the injection for further analysis and it took 10 minutes to complete the method.

Materials and Method for UV-visible Spectroscopy:

Instrument and Materials: The instrument utilized were JASCO model V630 and twin beam UV-visible spectrometer. Tolinaftate drug was obtained from Yarrow chemicals and Methanol used as solvent was HPTLC grade.

Selection of Media: Solubility and stability are key factors in media selection; the medicine must be equally soluble and stable in the medium of choice for a sufficient duration of time. In water, tolinaftate is essentially insoluble. Since tolinaftate is soluble in methanol, methanol has been chosen as the analytical solvent for this research.

Preparation of Standard Stock Solution: After dissolving a carefully measured 100 mg of tolinaftate in 5 ml of methanol, the volume was then made up to 100 ml to create a solution of 1 mg/ml.

Scanning: Between 200 to 400 nm, a number of concentrations – 2, 4, 6, 8 and 10 µg/ml were developed with previously mentioned stock solution. The 223 nm absorbance peak at 1.317 was selected for further research was shown in Fig. 11.

Preparation of Calibration Curve: A standard solution containing 1mg/ml of tolinaftate was made by mixing 50 mg of unadulterated tolinaftate in a solution of methanol of 50 ml. This solution was diluted with methanol to produce a working standard solution that contains 0.2-1.2 µg/ml of tolinaftate. The solution's absorbance at 257 nm was measured in comparison to a blank. All measurements of spectral absorbance were performed using a JASCO 1800 UV-visible spectrophotometer.

Pharmaceutical Formulation Sample Solution: You're using a sample of Dermiford Cream which contains 100 mg of tolinaftate (TF). It's placed in a 100 mL volumetric flask with 75 mL of methanol to begin dissolving the drug. While the flask was heated over a bath the mixture was been sonicated for fifteen minutes to completely melt the cream. A final volume of 100 ml of methanol is been added to the solution after sonication.

1000µg/ml stock solution was been made in order to make a 100µg/ml working solution. We took 1 mL of stock solution and diluted it to 10 mL total volume to get 100 µg/mL shown in Figure.

Forced Degradation Studies:

Acid Induced Degradation Study: To achieve a concentration of 100 µg/mL, 10 mg of tolinaftate that had been precisely weighed was added to a 10 mL volumetric flask, dissolved, and diluted with mobile phase. Five milliliters of this medication solution were measured precisely, then five milliliters of 0.01 HCl were added. For 10 minutes, the mixture was maintained at 15°C. After neutralizing the deteriorated solution with 0.01 N NaOH, the HPLC analyses were conducted under ideal chromatographic circumstances.

Base-induced Degradation Study: Measured precisely after adding 5 mL of 0.01 N NaOH to a solution containing 100 µg/mL of medication, the mixture was maintained at 25°C for 30 minutes. After neutralizing the deteriorated solution with 0.01 N HCl, the HPLC analyses were conducted under ideal chromatographic circumstances.

Oxidative Degradation Study: Five milliliters of a solution containing 100 µg/mL of the drug was measured precisely, and five milliliters of 3% v/v H₂O₂ were added. For twelve hours, this solution

was maintained at room temperature. Optimized chromatographic conditions were used to run the deteriorated solution *via* HPTLC.

Photolytic Degradation Study: For 4,8,12, and 24 hours, 100 mg of a typical medication was exposed to UV radiation in a UV chamber (Dolphin, India).10 mg of drug was precisely measured and dissolved in 10 ml of mobile phase in multiple volumetric flask at regular intervals to attain a concentration of 100µg/ml. Using HPTLC the resulting solutions were added for evaluating the degradation behavior.

Thermal Degradation Study: For 4,8,12, and 24 hours, 100 milligrams of the normal dosage were kept at 50°C. To reach a concentration of 1,000 µg/mL, 10 mg of the drug was carefully measured and mixed in 10 mL of mobile phase at regular intervals using different volumetric flasks. The resulting solutions wereinjected using HPLC to analyze the degradation behavior.

Method Validation: The optimized procedure was validated in accordance with ICH requirements.

Linearity: The correlation between an analytes concentration and the sensors response is determined by linearity. Tolinaftate stock solutions which ranged from 0.2 to 1.2µg/ml were made.

TABLE 1: LINEARITY DATA OF TOLNAFTATE

Sr. no.	Conc	Area I	II	III	IV	V		Mean	SD	%RSD
1	0.2	1325.5	1324.2	1326.3	1325.3	1326.6	1325.0	1325.4	0.866	0.065
2	0.4	2559.2	2559.9	2557.6	2558.6	2560.0	2560.1	2559.2	0.917	0.036
3	0.6	3780.6	3780.8	3781.0	3779.9	3780.8	3780.5	3780.6	0.39	0.01
4	0.8	5150.2	5149.5	5150.6	5150.8	5149.9	5150.0	5150.1	0.61	0.012
5	1	6357.9	6358.0	6357.9	6357.7	6357.0	6357.9	6357.7	0.1	0.002
6	1.2	7858.5	7858.9	7858.7	7859.0	7857.9	7858.0	7858.5	0.522	0.0066
Avg SD									0.5675	

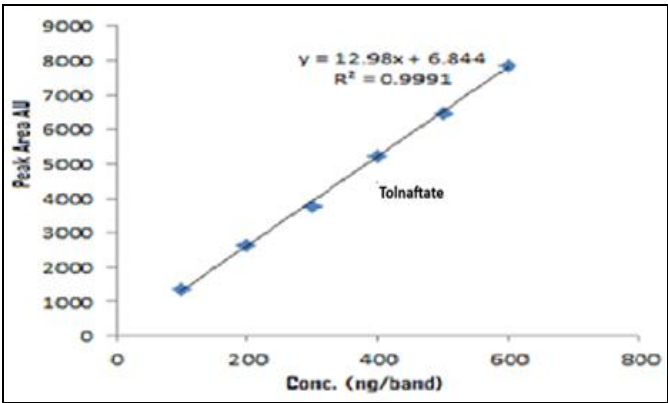


FIG. 2: LINEARITY GRAPH OF TOLNAFTATE

Each concentrations sample was administered in three separate instances and peak area was noted carefully. Using average peak area versus concentration, the curve was calibrated. A regression equation was performed, with a correlation coefficient (R²) > 0.999 which suggested very good linearity for the range selected. The data also corroborated that the method ensures a linear response for tolinaftate to quantify accurately in dosage forms containing varying concentrations of it in **Table 1** and **Fig. 2**.

Limit of Quantification (LOQ) and Limit of Detection (LOD): The method's sensitivity was evaluated using LOD and LOQ, which define the minimal amount of analyte that can be correctly recognized and determined respectively. These parameters are calculated using the formula of slope (S) of calibration curve and standard deviation of y-intercepts (σ) and formulae $LOD = 3.3 \times \sigma / S$ and $LOQ = 10 \times \sigma / S$. The method was suitable for trace analysis in pharmaceutical formulations or stability studies since the estimated LOD and LOQ values showed that it was sensitive enough to detect and quantify low levels of tolinaftate in **Table 3**.

Accuracy: The accuracy of the method was determined through recovery studies, which assess how closely the measured values agree with the actual values. This was carried out by spiking known amounts of tolinaftate (2, 4, and 6 $\mu\text{g/mL}$) with a fixed concentration of the sample solution (1 $\mu\text{g/mL}$). Each spiked solution was analyzed in triplicate, and the percentage recovery was calculated.

The recovery values in the results ranged between 98 % and 102% which is within the range of ICH which has been authorized. These results proved the method's accuracy and ability to extract the drug's true quantity from formulation without any disruption.

Precision: Recovery studies, which evaluate the degree of consistency between the measured and actual values, were used to ascertain the method's accuracy. To do this, a fixed concentration of the sample solution (1 $\mu\text{g/mL}$) was mixed with known amounts of tolinaftate (2, 4, and 6 $\mu\text{g/mL}$). The percentage recovery was calculated after each spiked solution was examined in triplicate. The recovery values in the results ranged between 98% and 102% which is within the range of ICH which has been authorized. These results demonstrated the method's accuracy and ability to extract the drug's true amount from the formulation with little loss or interruptions.

Robustness: The ability of the method to cope with minor, intentional adjustments to analytical parameters is examined by robustness. This was assessed by varying the flow rate from (0.9 and 1.1

mL/min), wavelength (254 and 256nm), and composition of the mobile phase ($\pm 2\%$ methanol) slightly. Under each modified condition, the sample solution and the standard solution (3 $\mu\text{g/mL}$) was examined. The assay results and percentage RSD values were contrasted with those acquired under typical circumstances. The peak area and retention time were not significantly altered in spite of the intentional changes, and the percentage RSD stayed within allowable bounds.

Analysis of in house Topical Solution: After carefully weighing 1ml of tolinaftate medication solution (10,000 $\mu\text{g/mL}$), it was put into a 10 ml volumetric flask. After incorporating the mobile phase, the mixture was sonicated for 15 minutes to estimate the amount tolinaftate present in the prepared solution.

In order to get a solution comprising 1,000 $\mu\text{g/mL}$ of tolinaftate, the solution was first mixed, diluted with mobile phase until the necessary level was obtained, then filtered through a sample filter. To modify the volume, 0.1 ml of this solution was extracted and then mobile phase was deposited into a 10 ml volumetric flask. The resultant solution has undergone three distinct HPTLC runs. In this in house topical solution, the weight % tolinaftate was calculated.

RESULTS:

HPTLC Method Development: A combination of Toulene, Chloroform, and Methanol resulted in the drugs desired separation from its breakdown products with the best retention period. Acetonitrile and water were used as mobile phase in the 3:1:1 v/v ratio since this mobile phase produced the best results. **Fig. 3** shows that tolinaftate was eluted at 0.27 minutes. 1 ml/min was the flow rate and 20 μl was the injection volume and ten minutes was the run time of the chromatography.

The drug exhibited absorption maxima at 255nm, according to UV spectra of a standard solution of tolinaftate, which was recorded in order to identify the proper wavelength. A well separated peak of tolinaftate from degradation peaks employing an optimal mobile phase was seen in the forced degradation sample example (acid hydrolysis, base hydrolysis and oxidation).

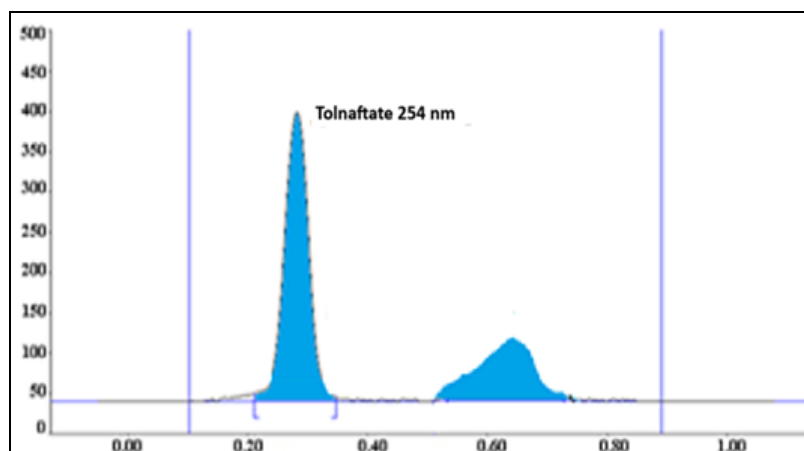


FIG. 3: HPTLC CHROMATOGRAM OF TOLNAFTATE STANDARD 100 µg/ML AT 245 NM

Forced Degradation Study:

Acid Induced Degradation Study: As shown by Fig. 4, the medication displayed breakdown within 10 minutes of contact with 0.01 N HCL at 15°C.

Analysis showed that during this short period of time in the presence of 0.01 N HCL and acidic conditions around 27.27% of the drug was degraded.

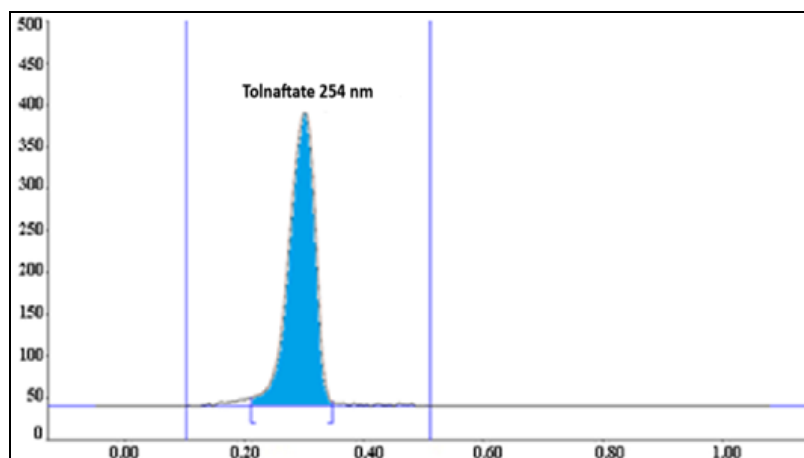


FIG. 4: CHROMATOGRAM SHOWING ACIDIC DEGRADATION OF TOLNAFTATE TABLET

Base-Induced Degradation Study: When 0.01 N NaOH was introduced at 25°C the drug showed significant disintegration after 30 minutes of treatment in Fig. 5. When 0.01 N NaOH was

introduced again it appeared that up to 24.05% of the entire drug has been degraded within 30 minutes at 25°C.

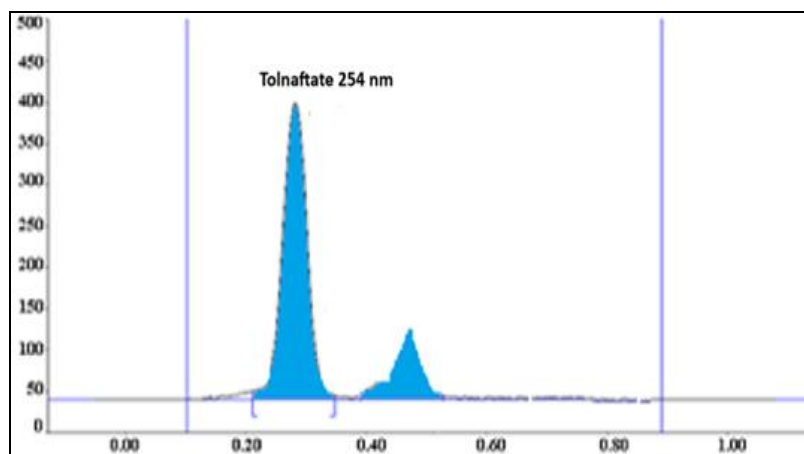


FIG. 5: CHROMATOGRAM SHOWING ALKALI DEGRADATION OF TOL TABLET

Oxidative Degradation Study: When the drug came into contact with the solution of H_2O_2 it showed a significant disintegration of 14.18% of its

initial quantity after keeping it for 12 hours at room temperature which is shown in **Fig. 6**.

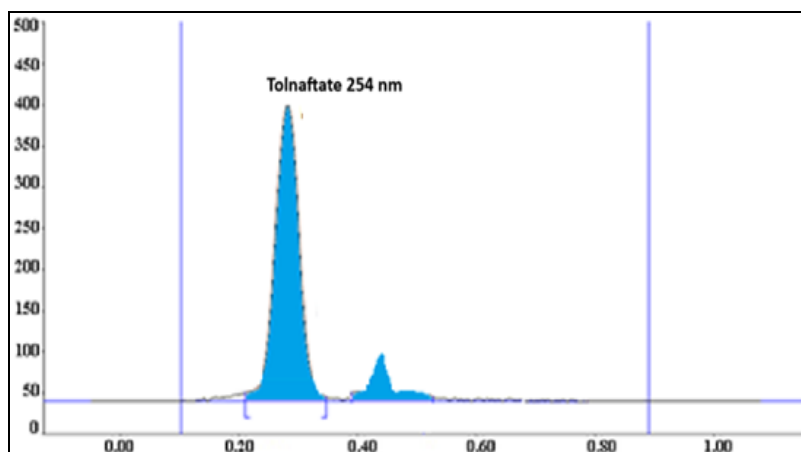


FIG. 6: CHROMATOGRAM SHOWING OXIDATIVE DEGRADATION OF TOLNAFTATE TABLET

Photolytic Degradation Study: The drug showed no photolytic degradation for 24 hours in **Fig. 7**. As

a result, the drug was shown to be photolytically stable.

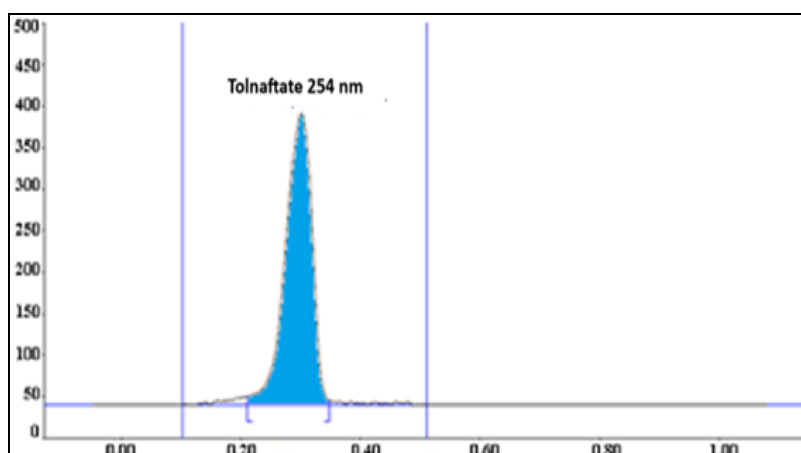


FIG. 7: CHROMATOGRAM SHOWING PHOTOLYTIC DEGRADATION OF TOLNAFTATE TABLET

Thermal Degradation Study: The drug was shown no degradation after being exposed to heat

for 24 hours in **Fig. 8**. The drug was therefore shown to be thermally stable.

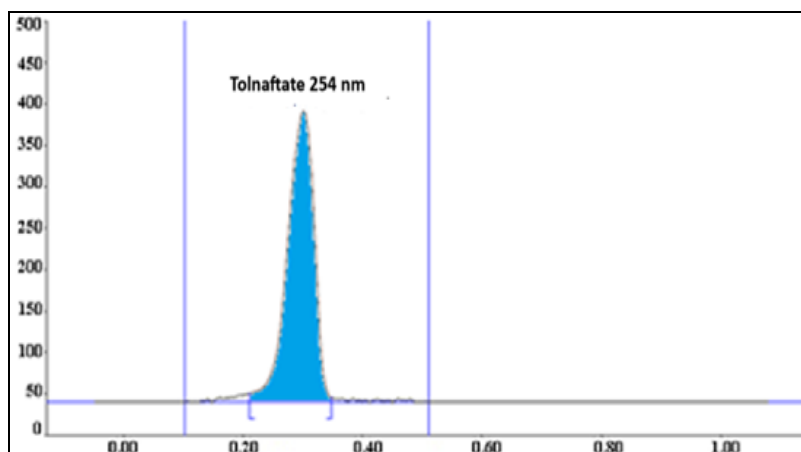
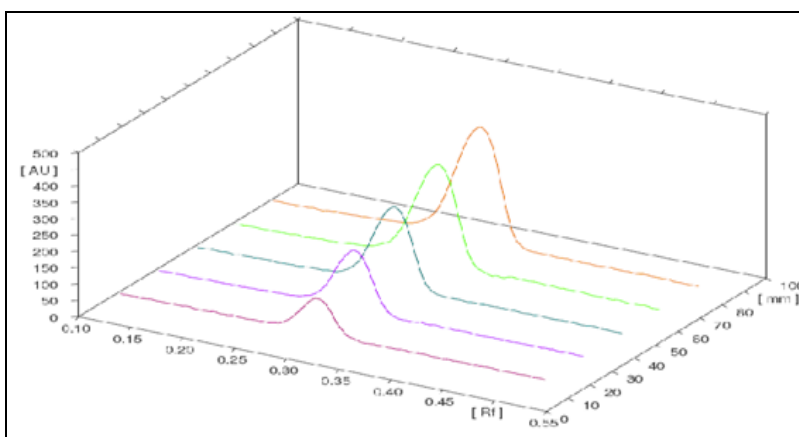
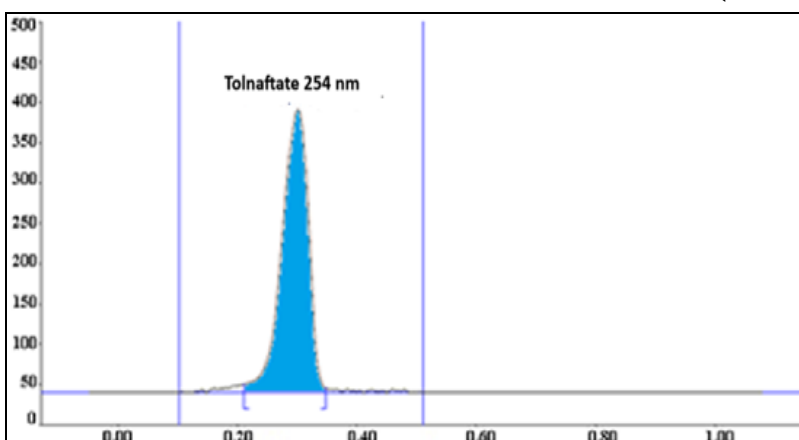
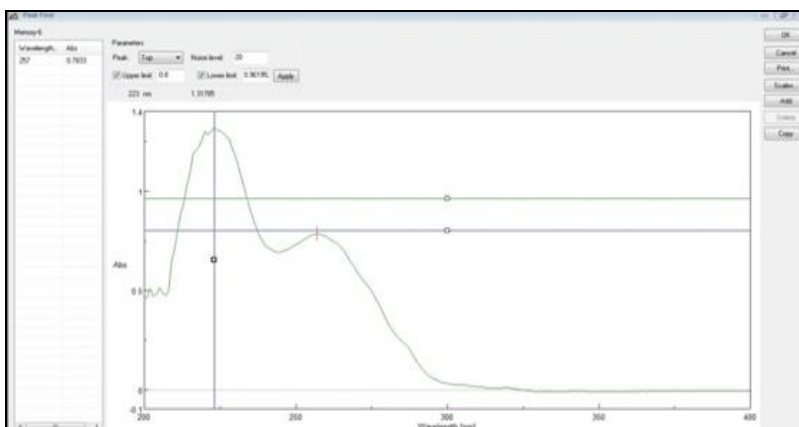
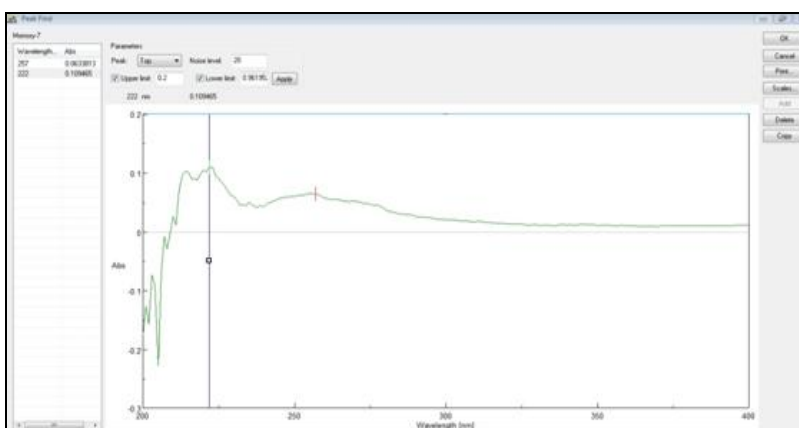


FIG. 8: CHROMATOGRAM SHOWING THERMAL DEGRADATION OF TOLNAFTATE TABLET

**FIG. 9: 2D CALIBRATION LINEARITY GRAPH OF DRUG TOLNAFTATE (100 TO 600 NG/ BAND)****FIG. 10: CHROMATOGRAM SHOWING UV DEGRADATION OF TOLNAFTATE TABLET****FIG. 11: STANDARD SOLUTION OF TOLNAFTATE SHOWING HIGHEST PEAK AT 223NM****FIG. 12: SAMPLE SOLUTION OF TOLNAFTATE SHOWING HIGHEST PEAK AT 222NM**

HPTLC Method Validation: The developed HPTLC method was evaluated against various validation parameters and it was found to be specific, as no interference was observed from the solvent, mobile phase, or other sample components in the detection of drug. System suitability parameters, such as peak symmetry ($T = 1.09$), resolution (< 2), and the number of theoretical plates ($N < 8000$), were found to be within acceptable ranges, including the optimized Chromatographic conditions. The method demonstrated a limit of detection (LOD) of $0.088\mu\text{g/ml}$ and limit of quantification (LOQ) of $0.421\mu\text{g/ml}$, indicating the procedure is sufficiently sensitive to detect and quantify tolinaftate at microgram levels. Linearity was observed in the concentration range of 0.276 to $6\mu\text{g/ml}$, with a correlation coefficient (R^2) of 0.9991 and linear regression equation $y = 12.98x + 6.844$. Accuracy wise, recovery percentage was within range. With the help of Interday and Intraday precision was studied at 3 concentrations. The actual range of % RSD of tolinaftate was < 2 in robustness study. For both modified and unchanged settings the tolinaftate w/w percentage ranged from 98.45% to 100.36% . The current study findings are displayed here and contrasted with earlier research.

Analysis of in-House Topical Solution: To estimate the tolinaftate content in the in-house topical solution, the validated HPTLC method was employed by comparing the responses of the test sample with those of the standard. The assay revealed that the formulation contained 98.28% tolinaftate. This result confirms that the method accurately measures tolinaftate without any interference from the excipients present in the formulation, demonstrating its reliability and specificity for routine analysis.

DISCUSSION: Several trials were carried out using different solvents and their varying proportions to identify the optimal mobile phase composition. The best chromatographic performance in terms of retention time, number of theoretical plates, peak symmetry, and resolution. This was accomplished using a mobile phase composed of toluene, chloroform, and methanol in a $3:1:1$ (v/v) ratio, delivered at a flow rate of 1.0 mL per minute . In the acid degradation study, tolinaftate was found to undergo complete

breakdown when a $1,000\mu\text{g/mL}$ solution of the drug was treated with 0.1 N hydrochloric acid and kept at 60°C for a period of eight hours. To generate suitable degradation products, the tolinaftate solution was initially refluxed with 0.01 N HCl at 40°C for eight hours, resulting in complete degradation. Subsequently, the drug solution ($1,000\mu\text{g/mL}$) was treated with 0.01 N HCl and maintained at 25°C for two hours. Even with reduced temperature and a shorter duration of exposure, the drug still underwent complete degradation. In further experiments, the exposure time was reduced to 30 minutes while keeping the acid concentration and temperature (0.01 N HCl at 25°C) constant, which led to significant degradation. Continuing with this approach, the drug solution was treated for 20 minutes under the same conditions, again showing considerable degradation. Finally, treatment for just 15 minutes at 25°C with 0.01 N HCl also resulted in noticeable degradation, confirming that tolinaftate is highly susceptible to acid hydrolysis even under mild conditions.

This resulted in notable degradation of the drug. To address this, the temperature and duration of exposure were reduced, while the acid concentration was kept constant. The study of base-induced degradation initially involved treating the drug solution with 0.1 N sodium hydroxide at 60°C for eight hours. After cooling, the solution was neutralized using 0.1 N hydrochloric acid. This condition led to complete degradation of the drug. Consequently, the experimental conditions were adjusted by reducing both the base concentration and temperature specifically, using 0.01 N NaOH and refluxing at 40°C for eight hours to obtain a suitable level of degradation.

However, this also resulted in complete drug breakdown. To optimize the conditions, the treatment was further modified by lowering the temperature and exposure time to 25°C and two hours, respectively, while maintaining the base concentration at 0.01 N . Under these conditions, significant drug degradation was observed. As a result, it was decided to shorten the exposure time while keeping the base concentration and temperature constant. In the oxidative degradation study, a drug solution with a concentration of $1,000\mu\text{g/mL}$ was exposed to 3% v/v hydrogen peroxide

at ambient temperature for a duration of six hours. Under these conditions, minimal degradation was observed. Therefore, the exposure time was extended to 12 hours at room temperature, which led to adequate drug degradation. As part of the photolytic degradation study, the drug was exposed to ultraviolet light inside a UV chamber under controlled conditions for a duration of 24 hours. Following suitable dilutions, no visible degradation products were detected under these conditions. As a result, the duration of exposure was gradually increased to 4, 8, 12, and 24 hours. Even after 24 hours of continuous UV exposure, no degradation products were observed, indicating that the drug is photolytically stable. In the thermal degradation study, the drug was exposed to a temperature of 500°C for the same time intervals 4, 8, 12, and 24 hours. No significant degradation was noted under these conditions, suggesting that the drug is thermally stable as well. Except for the degradation products formed under acidic conditions, all major degradation products of tolinaftate were successfully detected and characterized. The HPTLC method applied for validation was found to be straightforward, dependable, and effective. When compared with previously published methods, it was observed that some validation parameters in earlier studies were either incomplete or not clearly reported. The developed method, after thorough validation, proved to be reliable for accurately estimating tolinaftate, even when its degradation products are present.

CONCLUSION: A reversed-phase HPTLC-PDA method was successfully developed, offering a simple, rapid, sensitive, accurate, and precise analytical approach. Under optimized conditions, the method produced a well-defined symmetric peak and a high number of theoretical plates, along with satisfactory retention suitable for analysis. The method was validated following the ICH guidelines. It also addressed and included validation parameters that were either missing or not thoroughly reported in earlier studies. The validated method was effectively used to quantify tolinaftate in a topical formulation, even in the presence of its degradation byproducts.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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