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Development and Validation of RP-HPLC Method for Simultaneous Quantification of Tavaborole, Hydrocortisone, and Clindamycin Phosphate in Pharmaceutical Dosage Forms

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KEYWORDS

ICH guideline (Q2 R1); RP-HPLC; Tavaborole; Hydrocortisone; Clindamycin Phosphate and Validation

ABSTRACT:

This study introduces a novel reverse-phase high-performance liquid chromatography (RP-HPLC) method for concurrently quantifying Tavaborole, Hydrocortisone, and Clindamycin phosphate in diverse pharmaceutical formulations, including topical solutions, creams, and gels. The method employs the use of mobile phase involving 10 mM Ammonium acetate and Acetonitrile with Acetic acid, offering a sensitive and cost-effective approach. Validation studies demonstrate the method's accuracy, precision, and robustness, rendering it suitable for comprehensive analysis of combined dosage forms. Utilizing a Unisphere C18 column along with UV absorption detection at 225 nm, the analysis reveals retention times of 3.1 minutes for CLN, 5.1 minutes for HYN, and 6.1 minutes for TVB. The method exhibits excellent percentage recoveries for all compounds, ranging from 96% to 99%. Extensive validation includes assessments of linearity, selectivity, recovery, repeatability, LOD/LOQ values, and suitability in topical solution, cream, and gel placebo formulations. This cost-effective technique enables simultaneous and precise quantification of Tavaborole alongside Hydrocortisone and Clindamycin phosphate in various pharmaceutical compositions, underscoring its significance as a dependable tool for quality assurance and pharmaceutical research, offering both accuracy and efficiency.

1. INTRODUCTION

A newly identified oxoborole is a recognized class of boron-containing compounds that have shown comprehensive antifungal activity against various molds, fungi, and dermatophytes. Among these, Tavaborole (TVB), also known as 5-fluoro-1-hydroxy-3H-2,1-benzoxaborole (Fig. 1), received FDA approval in July 2014 as Kerydin for treating toenail onychomycosis infections.

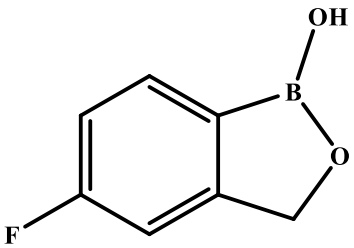

Fig. 1. Structure of Tavaborole

TVB works by inhibiting fungal cytoplasmic leucyl-tRNA synthetase, thereby hindering protein synthesis [1]. Onychomycosis is indeed a challenging fungal infection that affects the nails, primarily the toenails, but it can also occur in the fingernails, causes symptoms like nail thickening, discolouration, and detachment from the

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nail bed. It's commonly affected by dermatophytes and is more prevalent among older individuals, particularly diabetics, especially those with ulcers in the foot. Treatment options for onychomycosis, caused by dermatophytes, typically include both oral and topical medications, but the trouble lies inherent nature of the infection within the nail element and the limited penetration ability of drugs [2-4]. Tavaborole's lower molecular weight (152) and hydrophobic properties allow it to penetrate efficiently through the entire thickness of human nail plates [5, 6].

Acne vulgaris, a common inflammatory condition, tends to affect females more frequently than males [7, 8]. It is a recurring infection with long-lasting effects, impacting nearly everyone at some point in their lives [9]. Recent research suggests that combining multiple topical medications with different mechanisms of action leads to better outcomes compared to individual treatments [10]. Clindamycin phosphate (Fig. 2), a lincosamide-class antibiotic, is commonly prescribed for bacterial infections [11]. It exhibits a bacteriostatic effect and is effective against various bacterial strains responsible for infections can encompass both Gram-positive and Gram-negative bacteria, each with unique characteristics and susceptibilities [12]. When applied topically, Clindamycin targets Propionibacterium acnes on the skin, reducing inflammation and preventing the formation of new acne lesions, making it a valuable treatment option for managing acne vulgaris.

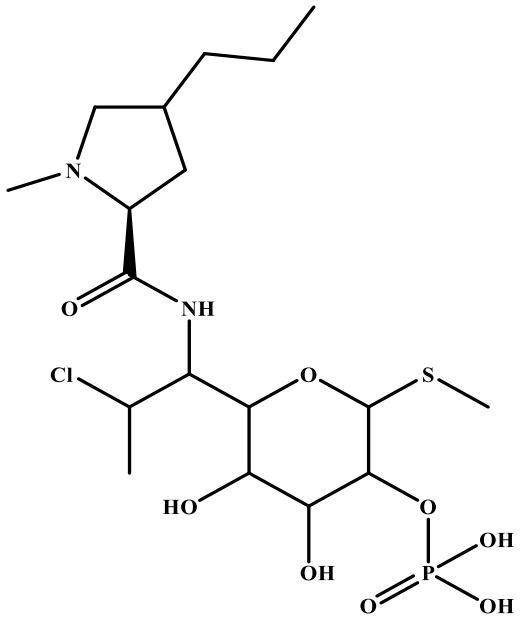

Fig. 2. Structure of Clindamycin Phosphate

Hydrocortisone (Fig. 3), also referred to as cortisol when acting as a hormone, is utilized for treating various conditions such as asthma, dermatitis, rheumatoid arthritis, adrenocortical insufficiency, adrenogenital syndrome and chronic obstructive pulmonary disease (COPD) [13,14]. It can be administered orally, topically through creams, or via injections, functioning as both an immune suppressant and an anti-inflammatory agent. Hydrocortisone acetate (HCA) is commonly applied topically to address skin disorders [15-16].

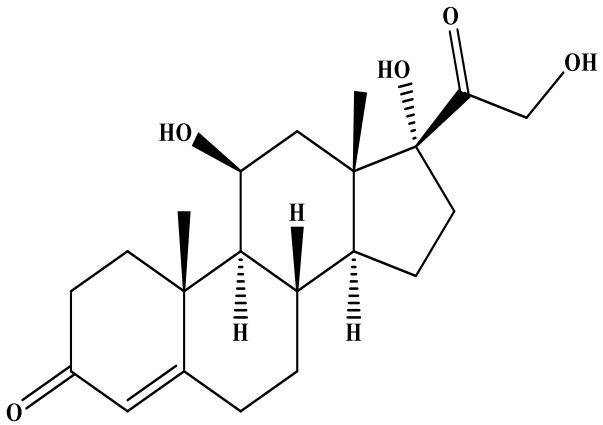

Fig. 3. Structure of Hydrocortisone

Existing literature indicates a shortage of analytical methods for accurately quantifying Tavaborole (TVB) in biological or pharmaceutical samples, with some studies focusing on in-vitro transungual permeation experiments and the characterization of degradation products [17, 18]. Additionally, a validated UV-spectrometric method has been established for quantifying Tavaborole [19]. Various analytical techniques have also been documented for quantifying Hydrocortisone and Clindamycin Phosphate separately and in combination with other medications [20-24].

Considering the recent approval of Tavaborole as an antifungal agent for Onychomycosis treatment, its combination with Hydrocortisone (an anti-inflammatory) and Clindamycin Phosphate (an antibiotic) presents a new approach not currently available in any pharmaceutical formulation. This combination holds promise for addressing dermatophyte infections by providing both anti-inflammatory and antibiotic effects in a single product.

The current research aim to develop and validate a simultaneous high-pressure liquid chromatography (HPLC) method using Tavaborole in combination with

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Hydrocortisone and Clindamycin Phosphate, and to assess its suitability across various placebo dosage forms sourced from the pharmaceutical industry.

2. OBJECTIVE

To develop a simple, efficient and validated simultaneous HPLC method to estimate the Tavaborole with Antibiotic and anti-inflammatory drugs.

3. MATERIALS AND METHODS

3.1 The analytically pure Active Pharmaceutical Ingredients (APIs) for Clindamycin phosphate, Hydrocortisone, and Tavaborole were obtained from Sigma-Aldrich.

S. No	Chemicals / Reagents	Grade	Company
1	Water	Ultrapure	Milli Q
2	Acetonitrile	HPLC	Honeywell
3	Methanol	HPLC	Honeywell

3.2. Instrumentation used:

The HPLC setup includes components such as a low-pressure gradient unit in the Thermo Scientific Dionex Ultimate 3000 RS pump, a WPS-3000TPS autosampler, a column compartment, and a PDA detector. Analytical data acquisition and processing were managed using a workstation equipped with Chromeleon software version 7.2. An ultrasonic bath sonicator was used consistently throughout the study.

3.3. Preparation of standard solution:

25 mg of CLN, HYN, and TVB standards (working) were weighed accurately and individually transferred into 25 mL glass volumetric flasks. They were dissolved using 1 mL of DMSO, followed by the addition of acetonitrile to reach a solution concentration of 1000µg/mL. Portions of the stock solutions were then properly diluted with a diluent (composed of water and acetonitrile in a 50:50 v/v ratio) to obtain working standards of 100µg/mL solutions for CLN, HYN, and TVB.

3.4. Calibration standards:

The calibration standards of the developed method were assessed by analysing eight different solutions within the 1.0–200.0 µg/mL range for all three analytes; CLN, HYN, and TVB.

3.5. Chromatographic conditions:

Different solvent combinations, including water, acetonitrile, and an ammonium acetate buffer, were experimented with. Unisphere C18 column (5 µm, 4.6 × 150 mm,) held at a temperature of 40±2 ° C. achieved chromatographic separation. The optimized mobile phase, comprising 10mM ammonium acetate and acetonitrile in a precise ratio, was employed for chromatographic separation with the addition of 1 mL of acetic acid. The flow rate of the mobile phase was consistently maintained at 0.4 mL/min throughout the chromatographic analysis to ensure optimal separation and resolution of the target compounds, with an injection volume of 50 µL and a run time of 8 minutes. Analysis was performed at 225 nm (Fig. 4) using the mobile phase as the diluent. Standard solutions of varying concentrations were prepared and stored in the auto sampler at 10±2°C. The mobile phase, prepared daily, underwent degassing using an ultrasonic bath sonicator and filtration through a 0.45µm membrane filter before use.

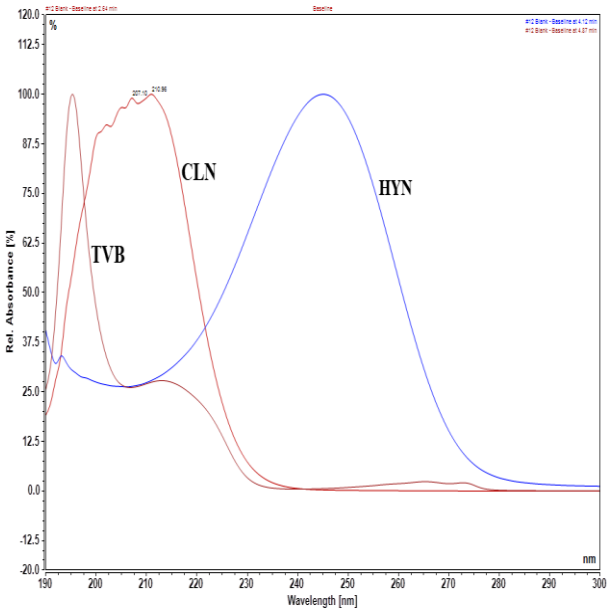

Fig. 4. Overlay spectra of TVB, CLN and HYN (50µg/mL)

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3.6. Method validation:
The validation of the RP-HPLC method included assessments for LOD, LOQ, linearity, precision, accuracy, and robustness, following the ICH Q2 (R1) guideline [25].

3.7. System suitability test:
The system suitability test included six consecutive injections of a standard mixture solution containing CLN, HYN, and TVB at a concentration of 50µg/mL. Various parameters of system suitability, such as retention time (tR), peak area, tailing factor (tf), theoretical plates, and resolution (R), were evaluated.

3.8. Linearity:
The analytical method linearity indicates its ability, within a specified range, to yield outcomes that are directly proportional to the analyte's concentration. Duplicate injections of differing volumes of standard mixture solutions containing CLN, HYN, and TVB were conducted to establish concentrations ranging from 1 to 200 µg/mL for each analyte. Linearity was evaluated by plotting peak areas against the corresponding drug concentrations using normal linear regression analysis. The slope, intercept (alongside their respective confidence intervals), and correlation coefficient (r^2) were computed and assessed.

3.9. Sensitivity:
The method's sensitivity was assessed using the Limit of Detection (LOD) and Limit of Quantification (LOQ). Specific concentrations of the analytes were injected to determine these limits. As per the ICH guideline, the signal-to-noise ratio for LOD should be greater than 3, and for LOQ, it should exceed 10.

3.10. Specificity:
The method's specificity was determined by injecting different samples, including a diluent, various placebo dosage forms, standard mixture solutions, and spiked placebo solutions. This was carried out to examine potential interference from excipients during the analytes' retention time.

3.11. Precision:
The evaluation of the developed method precision was evaluated through Intra-day and Inter-day precision analyses. In the Intra-day precision assessment, three replicates of three various concentrations (50, 100, and 200 µg/mL) were prepared by spiking the standard mixture solution into various placebos on the same day. The resulting peak areas were measured and expressed as percent relative standard deviation (% RSD). For the Inter-day precision study, the analysis was conducted on three Inter-days using various placebos with the specified concentrations of the three drugs in triplicate, and % RSD was calculated.

3.12. Accuracy:
The method accuracy was measured by adding three various amounts of CLN, HYN, and TVB to standard mixture solutions with concentrations of 50, 100, and 200 µg/mL, incorporated into various dosage forms of placebos (such as Topical Solution, Cream, and Gel). Recovery studies were conducted in triplicate, and both the recovery rates and % RSD for each drug were determined separately.

3.13. Robustness:
A standard mixture solution containing CLN, HYN, and TVB at a concentration of 50µg/mL was prepared and examined using two columns with comparable specifications. These columns were subjected to identical chromatographic and environmental conditions. The reproducibility of the results was subsequently evaluated as part of the analytical method's robustness study.

3.14. Application in placebo:
The proposed method's selectivity was examined by introducing all analytes into a standard mixture solution spiked into placebos of various dosage forms (like Topical Solution, Cream, and Gel). Subsequently, the recovery percentage of each component in the presence of matrices was measured. To conduct this, 1.0g of each placebo was weighed accurately and then individually transferred into separate 25 mL volumetric flasks. Then, 2.5 mL of a standard mixture solution with a concentration of 1000 µg/mL was added to each flask and thoroughly dissolved by vortexing. After this, the diluted the solution to volume with the diluent and filtered using Whatman No. 41 filter paper. Next, a quantity equivalent to 10 mL of this filtrate was again diluted to 10 mL with diluent to achieve final concentrations of 100 µg/mL for CLN, HYN, and TVB.

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4. RESULTS

4.1 Optimized condition for method development:

Following extensive experimentation, a variety of factors were explored to optimize the RP-HPLC method for the simultaneous detection of Clindamycin phosphate (CLN), Hydrocortisone (HYN), and Tavaborole (TVB). Among the parameters investigated, such as mobile phase compositions, columns, pH levels, and flow rates, the most favourable outcomes were achieved using the Unisphere C18 column (150 mm x 4.6 mm, 5µ) paired with a mobile phase consisting of 10mM Ammonium acetate and Acetonitrile in a 1:1 v/v ratio, complemented by 1mL of acetic acid. Detection occurred at 225 nm, and the rate of flow was set at 0.4 mL/min. Under these optimized conditions (Table-1), the retention times for CLN, HYN, and TVB were attained at 3.1, 5.1, and 6.1 minutes (Fig. 5), respectively. These parameters allowed for an efficient analysis with a short run time of 8 minutes. Through the refinement of the RP-HPLC method using the Unisphere C18 column and specific mobile phase composition, the analytical performance for the simultaneous determination of CLN, HYN, and TVB was significantly enhanced. This optimized approach, with its short run time and distinct retention times for each compound, establishes a dependable and effective method for quantifying these drugs in pharmaceutical formulations, especially topical solutions, creams, and gel-based placebos.

TABLE-1. Chromatographic Conditions

Parameters	Value
Flow rate	0.4ml/min
Sampler Temperature	10 °C
Column Temperature	40 °C
Injection volume	50µl
Detection wavelength	225nm

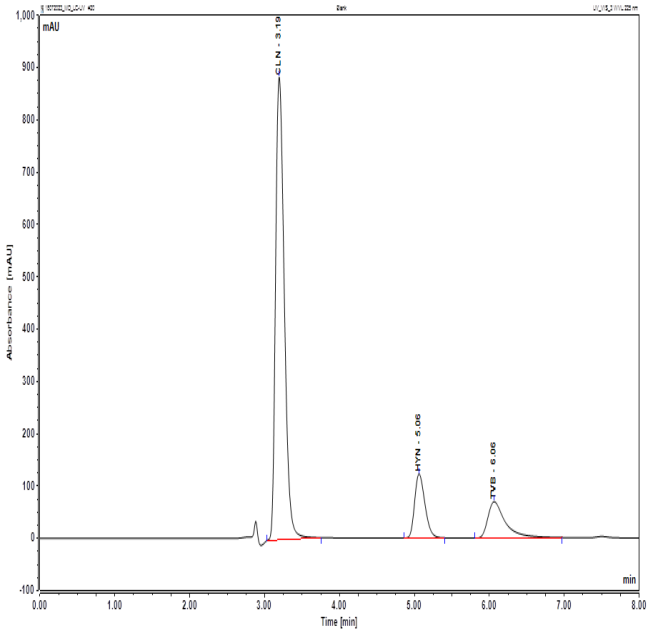

Fig. 5. Chromatogram obtained for CLN (3.1min), HYN (5.1) and TVB (6.1min) in a standard mixture solution (50µg/mL).

4.2. System suitability parameter:

The method, which was optimized, is considered acceptable, since the parameters of system suitability meet the criteria for acceptability, as indicated in Table-2.

TABLE-2. System Suitability for Clindamycin Phosphate, Hydrocortisone and Tavaborole

	System Suitability Parameters			
	Retention Time (Min)	**Resolution**	**Number of Theoretical Plates**	**Tailing Factor**
Clindamycin Phosphate				
Mean ± SD	3.15 ± 0.01	6.85 ± 0.03	7116 ± 6.42	1.27 ± 0.01
% RSD	0.14	0.44	0.09	0.90
Hydrocortisone				

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Mean ± SD	5.12 ± 0.00	2.86 ± 0.01	7707.6 ± 13.01	1.28 ± 0.06
% RSD	0.09	0.31	0.17	4.35
Tavaborole				
Mean ± SD	6.12 ± 0.01	*	16019.8 ± 247.82	1.14 ± 0.01
% RSD	0.12	NA	1.55	1.18

*Resolution for CLN and HYN were calculated based on TVB retention time.

4.3. Sensitivity:

The detection limit (LOD) and quantification limit (LOQ) were determined using signal-to-noise ratios of 3:1 and 10:1, respectively. For all analytes, CLN, HYN, and TVB, the LOD was determined to be 0.5µg/mL. Similarly, the LOQ for CLN, HYN, and TVB was found to be 1.0µg/mL, as presented in Table-3.

TABLE-3. Sensitivity

S. No.	Parameter	No. of Injection	µg/mL		
			CLN	HYN	TVB
1	LOD	3	0.5	0.5	0.5
2	LOQ	3	1.0	1.0	1.0

4.4. Specificity:

No interference from excipients was detected in any of the placebos for topical solution, cream, and gel when operating at the wavelength of 225 nm. The percentage of interference was less than 1% for all the drugs. This was confirmed by comparing the chromatograms of blank, placebo, and spiked sample preparation solutions, which revealed no interference of excipients with the peaks of CLN, HYN, and TVB. Thus, the method exhibited specificity for the tested placebos, as demonstrated in Fig. 6a, b, c and Fig. 7a, b, c. The results are presented in Table-4.

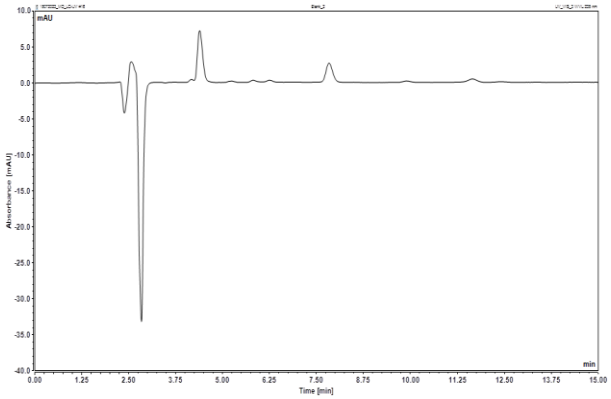

Fig. 6a. Chromatogram of Topical solution Placebo

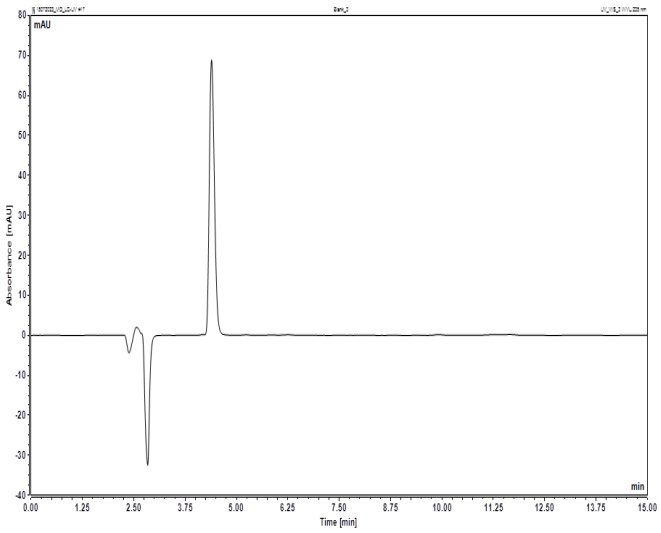

Fig. 6b. Chromatogram of Cream Placebo

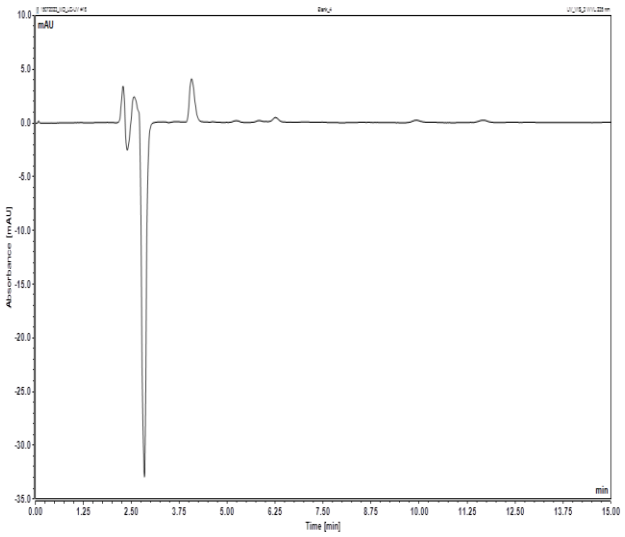

Fig. 6c. Chromatogram of Topical Gel Placebo

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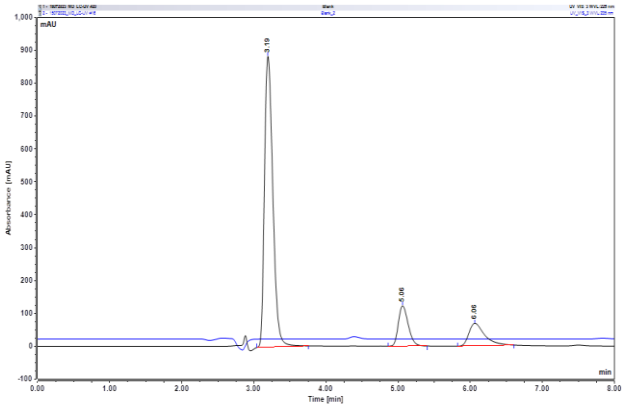

Fig. 7a. Overlaid chromatogram obtained for CLY, HYN and TVB in a spiked placebo of topical solution (100µg/mL)

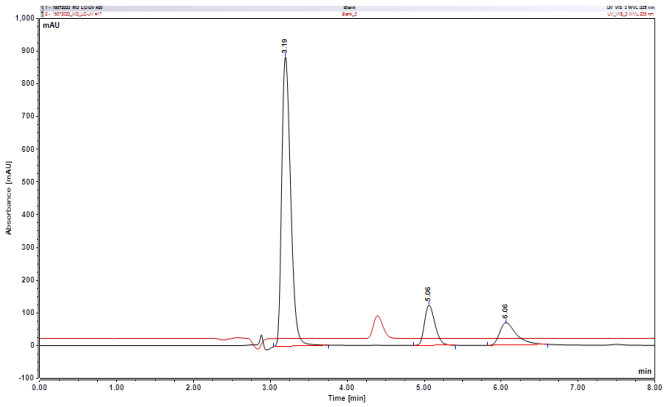

Fig. 7b. Overlaid chromatogram obtained for CLY, HYN and TVB in a spiked placebo of Cream (100µg/mL)

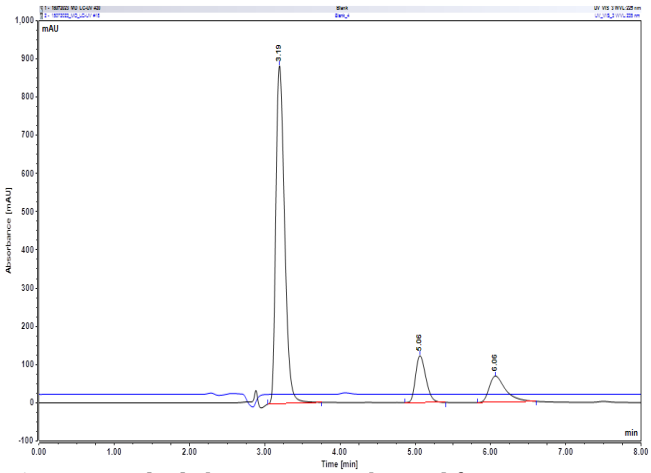

Fig. 7c. Overlaid chromatogram obtained for CLY, HYN and TVB in a spiked placebo of Gel (100µg/mL)

TABLE-4. Specificity for Clindamycin Phosphate, Hydrocortisone and Tavaborole

S. No	Name	No. of Injection	Area		
			CLN	HYN	TVB
1	Blank	1	Nil	Nil	Nil
2	Placebo	1	0	0	0
3	Standard	1	27.12	62.58	0.495
4	Spiked	1	26.31	61.95	0.475
5	Spiked	1	26.83	60.72	0.460
6	Spiked	1	26.03	59.50	0.451

4.5. Linearity:

A linear calibration curve was generated for CLN, HYN, and TVB across the 1-200 µg/mL range for each analyte, resulting in correlation coefficients of 0.999, 0.999, and 0.999 respectively.

4.6. Accuracy:

The method displays accuracy, with % recovery falling within the 98.0–102% range for CLN and TVB, and within the 95–103% range for HYN, as depicted in Table-5.

TABLE-5. Accuracy for Clindamycin Phosphate, Hydrocortisone and Tavaborole

Amount Drug Spiked (µg/mL)	Amount drug recovered	% Recovery	
		Mean ± SD	% RSD
Clindamycin Phosphate			
100.08	98.96	98.87 ± 0.12	0.12
Hydrocortisone			
100.60	98.14	97.55 ± 0.21	0.21
Tavaborole			
99.94	96.65	96.59 ± 0.23	0.24

4.7. Precision:

Repeatability and intermediate precision are quantified using % RSD, with values found to be < 5, demonstrating the method's high precision. Summary of results for both repeatability and intermediate precision can be found in Table-6.

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TABLE-6. Precision-Reproducibility for Clindamycin Phosphate, Hydrocortisone and Tavaborole.

Amount Drug Spiked (µg/mL)	Area Recovery	
	Mean ± SD	% RSD
Clindamycin Phosphate		
98.91	215.33 ± 0.28	0.13
Hydrocortisone		
98.91	418.48 ± 0.05	0.01
Tavaborole		
98.91	3.37 ± 0.13	3.92

4.8. Robustness:

During the robustness analysis, intentional alterations were made to the flow rate and mobile phase composition. The % RSD was observed to be less than 5%, indicating the method's robustness. These findings imply that even small variations in these parameters did not notably affect the selected factors.

4.9. Application of the Method:

The method described here was developed using a mobile phase comprising of 10mM Ammonium acetate and Acetonitrile, with the addition of 1mL Acetic acid, in a 1:1 ratio. This formulation resulted in a sensitive and cost-effective approach by significantly minimizing the use of organic solvents. Validation of the developed method was conducted separately using various pharmaceutical placebos, including topical solutions, gels, and creams. In these formulations, Clindamycin phosphate, Hydrocortisone, and Tavaborole were effectively separated, with retention times of 3.2 minutes, 5.1 minutes, and 6.1 minutes, respectively, demonstrating efficient separation of the three compounds.

5. DISCUSSION

After an extensive review of existing literature, no analytical method was identified for quantifying Tavaborole in combination with other drug products. Therefore, the current validated method was conducted as per ICH guidelines, which offers a unique and innovative approach in the field, enabling simultaneous analysis of Tavaborole alongside Clindamycin phosphate and Hydrocortisone. Overall, the reduced use of organic solvents, combined with the sensitivity and novelty of the method, makes it a valuable tool for future research and quality control analyses, especially in exploring drug combinations for treating fungal infections and related conditions.

6. CONCLUSION

A straightforward and rapid reverse-phase high-performance liquid chromatography (RP-HPLC) method has been devised successfully for concurrently determining Tavaborole, Hydrocortisone, and Clindamycin phosphate in various placebo dosage forms such as topical solutions, creams, and gels. With Tavaborole's recent approval for treating nail infections, this analytical method proves its ability to accurately assess Tavaborole, particularly when combined with anti-inflammatory and antibiotic medications, as evidenced by validation data. This negates the necessity for separate analyses and streamlines the comprehensive evaluation of the formulation's composition and stability. Overall, the development of analytical methods for simultaneously determining Tavaborole in combined dosage forms with anti-inflammatory and antibiotic medications marks a significant stride in pharmaceutical research and future quality.

7. ACKNOWLEDGEMENTS

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8. CONFLICT OF INTEREST

The authors state that there are no conflicts of interest concerning the conception and initiation of the paper, encompassing both financial and non-financial aspects.

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