RESEARCH ARTICLE

Development and validation of High-Performance Thin Layer Chromatographic methods for simultaneous determination of antibacterial pharmaceutical formulations containing Clindamycin phosphate

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ABSTRACT

The main objective of this research is to establish and authenticate the High-Performance Thin-Layer Chromatographic techniques for the simultaneous assessment of Clindamycin phosphate in combination with Tretinoin (formulation-1) and Clindamycin phosphate with Adapalene (formulation-2), both of which are antimicrobial combinations. In the developed HPTLC methods, for combination-1, the optimized mobile phase was a mixture of n-Hexane: Ethyl acetate: ACN: Methanol (5:2:2:1 by volume) with observed R_f values of 0.413 and 0.787 for Clindamycin phosphate and Tretinoin, respectively. Similarly, for combination-2, the mobile phase comprised n-Hexane: Diethyl ether: Dichloromethane: Acetic acid (4:4:2:0.1 by volume) with recorded R_f values of 0.407 and 0.559 for Clindamycin phosphate and Adapalene, respectively. The developed methods are successfully validated as per the regulatory guidelines. This approach proves to be significantly time-saving and cost-effective, rendering it suitable for routine analyses of the formulations containing selected antibacterial active pharmaceutical ingredients and commercial formulations.

Keywords: Antibacterial formulation, Adapalene, Clindamycin phosphate, HPLC, Tretinoin.

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INTRODUCTION

Microbial diseases are commonly observed disorders across all age groups. The integrity of both the environment and public health faces significant jeopardy from these diseases. Throughout human history, pathogenic bacteria, fungi, and viruses have consistently served as the primary culprits in the transmission of infectious diseases. Over time, a multitude of new antibiotics have been innovated to combat these threats. Antibiotic therapy, with its potent bactericidal effects and minimal harm to mammalian cells, has traditionally stood as the foremost treatment option for microbial infections. The availability of diverse antibiotic formulations further underscores the breadth of treatment options available to address a wide spectrum of diseases.[1]

Among adolescents and young adults, acne ranks as one of the most prevalent and enduring skin afflictions. The pathogenesis of acne involves various pivotal factors such as follicular hyperkeratinization, inflammation, external bacterial infiltration, and androgen-induced activation of sebaceous glands.[2] A multitude of oral and topical medications are commercially available for acne treatment, encompassing options such as tetracycline, erythromycin, Clindamycin phosphate (CLP), and Adapalene (ADA). The USFDA has sanctioned the utilization of CLP, an antibiotic belonging to the lincosamide class, for addressing anaerobic, streptococcal, and staphylococcal infections.[3] CLP is widely used as a prominent antibacterial drug, often in combination with Tretinoin (TRE). TRE is topically administered to address acne vulgaris, characterized by the presence of comedones, pustules, and papules.[4-5]

Apart from the aforementioned combination of CLP and TRE, ADA has also been introduced to the market, demonstrating efficacy in managing acne vulgaris alongside CLP. ADA, a derivative of vitamin A, functions to deter the accumulation of sebum, unclog pores, and facilitate the natural shedding of the outer skin layers.[6]

A thorough examination of the current literature reveals a plethora of methods documented for the analysis of CLP, TRE, and ADA, either individually or in various combinations with other drugs. These methods employ techniques such as HPLC, TLC, and UV spectrophotometry to determine the presence of the drug alongside other drugs.[7-11] To facilitate

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the development and validation of binary antibacterial formulations comprising CLP and TRE (formulation-1) and CLP and ADA (formulation-2), an advance thin-layer chromatographic technique (HPTLC) has been adopted. Chromatographic methods can be considered one of the most sensitive techniques, which are widely utilized for the determination of a number of active pharmaceutical ingredients along with respiratory steroids and a variety of phytoconstituents.[12-15] Nevertheless, it is notable that there is currently no validated HPTLC method specifically designed for the simultaneous assessment of CLP in combination with TRE and ADA as two separate combinations. In light of this gap, the key objective of current research is to develop and validate HPTLC analytical methods for these selected antibacterial pharmaceutical formulations.

MATERIALS AND METHODS

Chemicals and Reagents

CLP, TRE, and ADA were sourced from Dhamtech Pvt Ltd., Mumbai, India. All chemicals and reagents utilized in the method development and validation were of analytical grade, sourced from the local market. The commercial formulations containing both combinations were obtained from a registered medical supply store.

Instruments

HPTLC instrument (Camag, Switzerland) with a specific software named Vision CAT and a sample applicator named Linomate V, were employed alongside a TLC densitometric scanner (Camag, Switzerland), as a core equipment for overall method development and validation.

HPTLC Methods for Selected Combinations

Method for preparation of standard solutions

To achieve a final concentration of 1000 µg/mL for each drug, precise amounts of CLP and TRE were weighed and dissolved in methanol. Similarly, standard solutions of CLP and ADA were prepared by accurately weighing both APIs, with a final concentration of 1000 µg/ml being attained in methanol and dimethyl sulfoxide, respectively, in a ratio of 80:20.

Method for TLC of selected combinations

Various ratios of mobile phases were tested to attain significant Rf values for CLP and TRE, respectively. A similar approach was employed for the second combination, CLP and ADA. The optimization of the mobile phase and other chromatographic parameters for method development and validation relied on the results of multiple TLC trials conducted to further refine the HPTLC methods for both combinations.

Procedure for HPTLC method development for both the combinations

Aluminum 60 F254 (20 x 10) pre-coated plates with a thickness of 0.2 mm were obtained from Merck in Darmstadt, Germany. The plates were subjected to prewashing with methanol and activation for five minutes at 60°C before the chromatographic procedure commenced. The spotter, an automated TLC sampler named Linomate V, was operated for the application of samples. The bandwidth of 7 mm was maintained consistently throughout. For the development of chromatograms, twin trough glass chambers measuring 20 by 10 cm were utilized. The chamber saturation time was optimized to 20 minutes at 30°C with 22% relative humidity for the optimized mobile phase in both combinations. The mobile phase ratio for both CLP and TRE was optimised as (5:2:2:1 v/v/v/v) n-Hexane: Ethyl acetate: ACN: Methanol, with an overall volume of 10 ml. Similarly, the optimal mobile phase for CLP and ADA was determined to be (4:4:2:0.1 v/v/v/v) n-Hexane: Diethyl ether: Dichloromethane: Acetic acid. The drugs were scanned at appropriate wavelengths using the densitometric scanner, employing slit widths of 6.0mm x 0.45mm and scan speeds of 10mm/s. The detection wavelength was optimized at 241nm for both combinations. Peak regions on each plate were evaluated using linear regression.

Procedure for Analytical Method Validation of Developed HPTLC Methods

Linearity

The linearity was evaluated by spotting CLP and TRE at five different concentrations ranging from 12 to 120 ng/spot and 1 to 5 ng/spot, respectively. Similarly, for CLP and ADA, various concentrations in the range of 10 to 50 ng/spot and 1 to 5 ng/spot were measured, respectively.

Accuracy

Accuracy studies were undertaken to assess the recovery of the standards after spiking the specified concentrations of standard into pre-analysed commercial formulations for both combinations. The accuracy determination involved adding a predetermined amount of respective standards, which were spiked at three different levels (50, 100, and 150%) to the respective sample solutions. This procedure was applied to both selected antibacterial combinations, and overall percentage recoveries were calculated for each combination.

Precision

The developed method's intraday and interday precision were measured as a percentage of Relative Standard Deviation (RSD). Assessments were conducted on six separate days for intraday precision and six different days for interday precision. Furthermore, repeatability was also conducted for both combinations under the optimized experimental conditions of HPTLC methodologies for six replicates each. The procedure outlined above was followed for the simultaneous assessment of CLP and TRE, followed by CLP and ADA, respectively.

Robustness

Small changes in optimized HPTLC experimental conditions were carried out to evaluate the robustness of the developed methods. These includes change in mobile phase ratio, adjustment of chamber saturation time, and modifying variety of volumes of mobile phase.

Limits of detection and limit of quantification

The LOD and LOQ were determined by utilizing blank signals with the respective slopes of the calibration curves for both combinations. The procedure outlined above was adhered to for the simultaneous determination of CLP and TRE, followed by CLP and ADA, respectively, for the respective calculations of LOD and LOQ for both combinations.

Procedure for Application of the Developed HPTLC Methods for Commercial Formulation Analysis of CLP and TRE (Formulation 1) and CLP and ADA (Formulation 2)

The cream-based commercial formulation samples of CLP and TRE were prepared by dissolving a sufficient quantity of formulation in methanol, thoroughly shaking the mixture, and heating it for five minutes. Similarly, the cream-based formulation sample of CLP and ADA was prepared by dissolving a sufficient quantity of formulation in a mixture of methanol and dimethyl sulfoxide in a ratio of 80:20, respectively. Furthermore, both sample solutions were filtered using Whatman filter paper No. 41, and the HPTLC conditions described earlier were applied.

RESULTS AND DISCUSSION

Considering various parameters such as mobile phase selection with different solvent ratios, chamber saturation time, temperature, migration distance, sample application rates, and scanning speed, along with wavelength, play a crucial role and can be deemed essential in the overall optimization of the HPTLC method, followed by method development and validation. Taking into account all the aforementioned important parameters, and after conducting a variety of trials, n-hexane: ethyl acetate: ACN: Methanol (5:2:2:1 v/v/v/v) was chosen as the optimized mobile phase for the determination of CLP and TRE. Consequently, it was demonstrated that this solvent solution was suitable for the simultaneous assessment

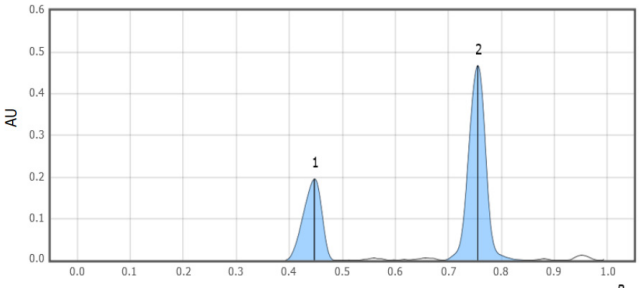

Figure 1: CLP (1) and TRE (2) HPTLC chromatogram using optimised mobile phase (Combination 1)

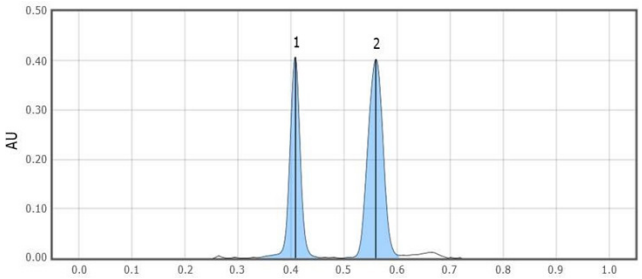

Figure 2: CLP(1) and ADA (2) HPTLC chromatogram using optimized mobile phase (Combination 2)

Table 1: Optimized chromatographic conditions for CLP + TRE (combination 1) and CLP+ ADA (combination 2)

Parameters	*Chromatographic condition*
Stationary Phase	Aluminium Plate Pre-coated with Silica Gel 60 F_{254}
Mobile phase	For combination 1 Hexane: ACN: Ethyl Acetate:methanol (5:2:1:2) v/v/v/v For combination 2 Hexane: Diethyl ether: Dichloromethane: acetic acid (4:4:2:0.2) v/v/v/v
Chamber saturation time in minutes	20
Temperature	Room temperature
Migration distance in mm	80
Application Parameters	
Application rate in ml/sec	100
Bandwidth in mm	6
Distance between tracks in mm	11.6
Distance between the plate edge in mm	15
Distance from the bottom of the plate in mm	15
Scanning parameters	
Slit dimension	4.00 × 0.30 mm
Scanning speed in mm/sec	20
Detection wavelength in nm	241
Lamp	Deuterium lamp

Table 2: Results of recovery studies of Combination 1 and Combination 2

Drug	*Level %*	*Amt of sample (standard)*	*Amt of spiked (ng/band)*	*Total amt*	*Amt of recovered*	**Avg of % Recovery ± SD*
Combination 1 - CLP+ TRE						
CLI	50	72	36	48	48.27	100.57 ± 0.34
	100	72	72	84	83.91	99.89 ± 0.56
	150	72	108	120	122.12	101.77 ± 0.68
TRE	50	1.5	0.75	1	1.00	99.85 ± 0.97
	100	1.5	1.5	1.75	1.78	101.60 ± 0.54
	150	1.5	2.25	2.45	2.46	100.50 ± 0.87
Combination 2 - CLP+ ADA						
CLI	50	10	5	15	14.92	99.46± 0.54
	100	10	10	20	20.12	100.6± 0.94
	150	10	15	25	24.78	99.12± 1.123
ADA	50	1	0.5	1.5	1.52	101.33± 0.69
	100	1	1	2	1.97	98.5± 0.87
	150	1	1.5	2.5	2.54	101.6± 0.94

*n=3

Table 3: Results of Robustness testing for both the selected combinations

S. No.	*Parameters combination 1*	*%RSD* [a]	
		CLP	*TRE*
1	Mobile phase composition Hexane: ACN: Ethyl Acetate: Methanol		
	1.(5:2:1:2) v/v/v/v	1.71	1.94
	2.(5.1:2:0.8:2) v/v/v/v	1.79	1.27
	3.(4.9:2:1.1:2) v/v/v/v	1.63	1.37
2	Mobile phase Volume		
	10 mL	1.29	1.10
	15 mL	1.01	1.19
	20 mL	1.43	1.36
3	Saturation time		
	20 minutes	0.79	0.98
	25 minutes	1.03	0.82
	30 minutes	0.76	0.98
Parameters - Combination 2		CLP	ADA
1	Mobile phase composition Hexane: Diethyl ether: Dichloromethane: acetic acid		
	1. (4:4:2:0.2) v/v/v/v	1.23	0.79
	2. (3.9:4.1:2:0.2) v/v/v/v	0.97	1.11
	3. (4.1:3.9:2:0.2) v/v/v/v	0.87	0.92
2	Mobile phase Volume		
	1. 10 mL	1.23	1.42
	2. 15 mL	1.41	0.97
	3. 20 mL	1.03	1.34
3	Saturation time		
	1. 20 minutes	1.65	1.19
	2. 25 minutes	1.38	0.68
	3. 30 minutes	1.29	0.74

[a] – n=3

Table 4: Results of Validation parameters for newly developed HPTLC methods for both the combinations

Parameters	*Combination 1 - CLP+ TRE*		*Combination 2 - CLP+ ADA*	
	CLP	*TRE*	*CLP*	*ADA*
Range (ng/band)	12-120	1-5	10-50	1-5
Slope	3.078	1.058	0.021	0.031
Intercept	13.907	10.01	0.023	0.033
Correlation coefficient $(R)^2$	0.9995	0.9997	0.999	0.9991
Accuracy (% Recovery ±SD)	100.74 ± 0.526	100.65 ± 0.793	100.74 ± 0.526	100.65 ± 0.406
LOD (ng/band)	3.46	0.23	2.59	0.27
LOQ (ng/band)	11.42	0.77	8.54	0.89
Precision (%RSD, n = 6)				
Intraday	1.18	0.59	0.56	0.94
Interday	1.69	0.79	0.87	1.89
Repeatability (%RSD, n = 6)	1.74	1.04	1.39	0.941
Robustness (%RSD) (n = 3)				
Mobile phase composition	1.71	1.52	1.02	0.94
Mobile phase volume	1.24	1.21	1.22	1.24
Chamber saturation time	0.86	0.92	1.44	0.87

Table 5: Application of the developed methods for simultaneous determination of both the selected combinations

Combinations	*Name of drug*	*Label claim* (mg/mixture)*	*Amount found (mg/mixture)*	*% Label claim (mg/mixture)**
Formulation 1	CLP	12	11.51	95.91 ± 0.23
	TRE	0.25	0.24	96.01 ± 0.36
Formulation 2	CLP phosphate	10	9.513	95.13 ± 0.45
	ADA	1	0.967	96.72 ± 0.34

*- n=3

of TRE and CLP. The HPTLC chromatogram for CLP and TRE is presented in Figure 1.

Similarly, the TLC method underwent initial optimization prior to the development of the HPTLC method for the second combination, i.e. a mixture of CLP and ADA. Following experimentation with various combinations of mobile phase ratio, it was determined that diethyl ether, dichloromethane, and acetic acid in the ratio of 4:4:2:0.1 v/v/v/v, would facilitate sufficient migration of the compounds on the prepared TLC plates. Subsequently, this solvent solution was proven to be suitable for the simultaneous assessment of CLP and ADA on separate plates using HPTLC as well. Figure 2 illustrates the HPTLC chromatogram for both ADA and CLP.

The optimized chromatographic conditions for both selected combinations are detailed in Table 1 as represented below:

Both developed HPTLC methods underwent successful validation in accordance with the regulatory guidelines of analytical method validation. The results of the accuracy studies for both combinations are presented in Table 2.

In accordance with the procedure, the developed HPTLC methods were also assessed for their robustness for both combinations, and the findings of the robustness testing were presented in Table 3.

An illustration of the results of several validation parameters is provided in Table 4 as presented below:

The developed and established methods were successfully utilized to determine CLP+ TRE (Combination 1) and CLP+ ADA (Combination 2) in commercially available formulations. The results of the analysis for both combinations of commercially available formulations are presented in (Table 5).

CONCLUSION

Accurate and sensitive HPTLC methods were developed and successfully validated for quantifying two binary pharmaceutical formulations containing CLP as a common active pharmaceutical ingredient. The results demonstrate the adequacy of the developed methods for routine analysis of CLP with TRE and with ADA in both their pure and commercial formulations. The findings of this validation process indicate that the proposed method is not only less time-consuming but also holds significant utility within the pharmaceutical industry for routine analyses. This methodological approach contributes to addressing the specific need for validated HPTLC methods for CLP in combination with TRE and ADA, thereby enhancing the analytical capabilities of these antibacterial formulations.

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REFERENCES

1. Fishbein SR, Mahmud B, Dantas G. Antibiotic perturbations to the gut microbiome. Nature Reviews Microbiology. 2023;21(12):772-88. DOI: 10.1038/s41579-023-00933-y
2. Bharti S, Vadlamudi HC. A strategic review on the involvement of receptors, transcription factors and hormones in acne pathogenesis. Journal of Receptors and Signal Transduction. 2021;41(2):105-16. DOI: 10.1080/10799893.2020.1805626
3. Guardabassi L, Apley M, Olsen JE, Toutain PL, Weese S. Optimization of antimicrobial treatment to minimize resistance selection. Antimicrobial Resistance in Bacteria from Livestock and Companion Animals. 2018;637-73. DOI: 10.1128/microbiolspec. ARBA-0018-2017
4. Gong J, Zhang D, Ran Y, Zhang K, Du S. Solvates and polymorphs of Clindamycin phosphate: Structural, thermal stability and moisture stability studies. Frontiers of Chemical Science and Engineering. 2017;11:220-30. DOI: 10.1007/s11705-017-1624-4
5. Ascenso A, Ribeiro H, C Marques H, Oliveira H, Santos C, Simões S. Is TRE still a key agent for photoaging management Mini-Reviews? in Medicinal Chemistry. 2014;14(8):629-41. DOI: 10.2174/1389557514666140820102735
6. Shroot B, Michel S, Galderma FC, Antipolis S. Pharmacology and chemistry of ADA. Journal of the American Academy of Dermatology. 1997;36(6):S96-103. DOI: 10.1016/s0190-9622(97)70050-1
7. Sarfraz S, Hussain S, Javed M, Raza A, Iqbal S, Alrbyawi H, Aljazzar SO, Elkaeed EB, Somaily HH, Pashameah RA, Alzahrani E. Simultaneous HPLC determination of Clindamycin phosphate, Tretinoin, and preservatives in gel dosage form using a novel stability-indicating method. Inorganics. 2022;10(10):168. DOI: 10.3390/inorganics10100168
8. Barazandeh Tehrani M, Namadchian M, Fadaye Vatan S, Souri E. Derivative spectrophotometric method for simultaneous determination of Clindamycin phosphate and Tretinoin in pharmaceutical dosage forms. DARU Journal of Pharmaceutical Sciences. 2013;21:1-7. DOI: 10.1186/2008-2231-21-29
9. Dobričić V, Bubić-Pajić N, Marković B, Vladimirov S, Savić S, Vuleta G. Development and validation of an LC-MS/MS method for the determination of Adapalene in pharmaceutical forms for skin application. Journal of the Serbian Chemical Society. 2016;81(10):1171-81. DOI: 10.2298/JSC160215066D
10. Martins LA, Meneghini LZ, Junqueira CA, Ceni DC, Bergold AM. A simple HPLC-DAD method for determination of Adapalene in topical gel formulation. Journal of chromatographic science. 2011;49(10):796-800. DOI: 10.1093/chrsci/49.10.796
11. Tashtoush BM, Jacobson EL, Jacobson MK. A rapid HPLC

method for simultaneous determination of Tretinoin and iso Tretinoin in dermatological formulations. Journal of pharmaceutical and biomedical analysis. 2007;43(3):859-64. DOI: 10.1016/j.jpba.2006.08.027

12. Ahmad S, Mujawar T, Batewal B, More P, Gaikwad A, Chumbhale D, Tare H. RP-UHPLC and HPTLC method development and validation for analysis of andrographolide from herbal hepatoprotective formulation. International Journal of Pharmaceutical Quality Assurance. 2023;14:96-104. DOI: 10.25258/ijpqa.14.1.17
13. Chepyala S, Medidi S, Malik JK. Development of a Robust and Reliable RP-HPLC Method for the Estimation of Finerenone in Tablet Dosage Form. International Journal of Drug Delivery Technology. 2024;14(2):703-708. DOI: 10.25258/ijddt.14.2.15
14. Patil MA, Godge RK, Dhamak KV, Mhaske SB. Simultaneous Estimation of Montelukast and Doxofylline in Bulk Drug and Tablet Dosage Form by UHPLC Method. International Journal of Pharmaceutical Quality Assurance. 2024;15(1):106-109. DOI: 10.25258/ijpqa.15.1.16
15. Patel S, Soni P, Omray LK. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Gliclazide in Bulk and Tablet Formulation. International Journal of Drug Delivery Technology. 2023;13(2):483-487. DOI: 10.25258/ijddt.13.2.05