Int. J. Sci. R. Tech., 2026 3(5)
A Multidisciplinary peer-reviewed Journal
www.ijsrtjournal.com [ISSN: 2394-7063]

Original Article

Development And Validation Of RP HPLC Method For The Simultaneous Estimation Of Clindamycin Phosphate And Benzoyl Peroxide In Pure And Dosage Forms

M. Venkataramana*, P. Sri Anjali, D. Shivaparameshwari, M. Navya Sri, M. Sri Vidhya, Daraksha Anjum

Department of Chemistry, Surabhi Dayakar Rao College of Pharmacy, Rimmanaguda, Gajwel, Siddipet, Telangana-502312.

ABSTRACT

A simple, rapid, and reliable reverse-phase high-performance liquid chromatography (RP-HPLC) method was successfully developed for the simultaneous estimation of Clindamycin phosphate and Benzoyl peroxide in pharmaceutical dosage forms. Chromatographic separation was achieved using a Phenomenex ZorbaxC18-SB column with a mobile phase of phosphate buffer (pH 4.5) and acetonitrile (45:55 v/v) at a flow rate of 1.2 ml/min, resulting in well-resolved peaks with retention times of 2.923 min and 3.939 min, respectively. The method demonstrated satisfactory system suitability parameters, including acceptable tailing factors and theoretical plate counts. Validation studies confirmed excellent linearity with correlation coefficients greater than 0.999 for both drugs. Precision and intermediate precision studies showed %RSD values within acceptable limits, while accuracy studies indicated mean recoveries close to 100%. Sensitivity was established through low LOD and LOQ values, and robustness studies confirmed the reliability of the method under slight variations in chromatographic conditions. Overall, the validated RP-HPLC method proved precise, accurate, sensitive, and robust, making it suitable for routine quality control analysis of Clindamycin phosphate and Benzoyl peroxide in bulk and pharmaceutical formulations.

Keywords: Clindamycin phosphate, Benzoyl peroxide, RP-HPLC, Method development, Validation, ICH guidelines.

INTRODUCTION

Pharmaceutical analysis ensures drug safety, efficacy, and quality throughout development using advanced techniques like High-Performance Liquid Chromatography (HPLC)[1,2,3]. Reverse Phase HPLC (RP-HPLC) is widely used to analyze pharmaceutical combinations, such as the acne treatment Clindamycin and Benzoyl Peroxide[4,5]. Simultaneous estimation of these drugs improves laboratory efficiency and reduces analysis time and solvent consumption[6]. However, this requires careful method development to manage challenges in polarity differences, analyte instability, and potential excipient interference[7]. The process involves optimizing chromatographic parameters to achieve well-resolved peaks with high reproducibility. This literature review reveals that Mandava et al[8] reported developed and validated an RP-HPLC method for simultaneous estimation of Clindamycin, Metronidazole, and Clotrimazole. The method used a C18 column with a flow rate of 1 mL/min and detection at 238 nm. The drugs were separated within 5 minutes, and the method showed good accuracy and precision with Flow rate is1ml/min and Run time is 5 min. Chemically Clindamycin is 7-chloro-6,7,8-trideoxy-6-{[4-(methylsulfonyl) phenyl] amino}-1-thio-L-threo-α-D-galacto-octopyranoside [9,10] having Antibiotic property and Benzyl peroxide is used as topical retinoid.

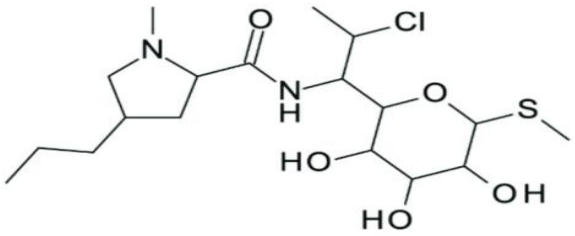

Figure 1: Structure of clindamycin

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

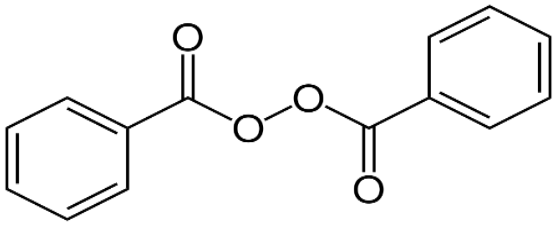

Figure 2 : Structure of Benzyl Peroxide

MATERIALS AND METHODS:

HPLC grade acetonitrile (Qualigens) and methanol (Rankem) were used in this study, and HPLC was used with auto sampler and PDA detector during the process.

Optimized Method conditions:

Instrument used : HPLC equipped with auto sampler and PDA detector

Temperature : Ambient

Column : Phenomenex Zorbax C_{18}-SB, (150×4.6mm, 3µm)

Buffer : Phosphate buffer (pH-4.5)

Mobile phase : 45% Phosphate buffer: 55% ACN

Flow rate : 1.2 ml per min

Wavelength : 220 nm

Injection volume : 10 µl

Run time : 8 min.

Method Validation: Method validation is performed according to guidelines such as those provided by ICH.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels; repeatability, intermediate precision and reproducibility.

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Assay:

Standard Solution Preparation: Accurately weigh and transfer 4mg of Clindamycin phosphate and 20mg Benzoyl peroxide working standard into a 20ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**,** Further pipette 0.6ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents (12ppm Clindamycin phosphate &6 0ppm Benzoyl peroxide).

Linearity: A stock solution was prepared by accurately weighing 4 mg of Clindamycin phosphate and 20 mg of Benzoyl peroxide into a 20 mL volumetric flask, dissolving with diluent, and making up the volume to the mark. For linearity studies, 0.2, 0.4, 0.6, 0.8, and 1.0 mL aliquots of the stock solution were transferred separately into 10 mL volumetric flasks and diluted to volume with diluent to obtain concentrations of 4, 8, 12, 16, and 20 ppm of Clindamycin phosphate and 20, 40, 60, 80, and 100 ppm of Benzoyl peroxide, respectively.

RESULTS AND DISCUSSION:

Optimized chromatographic conditions:

The optimized HPLC method used a flow rate of 1.2 ml/min, injection volume of 10µL and run time of 8 min . The analysis was performed using and HPLC system equipped with an auto sampler and PDA detector.

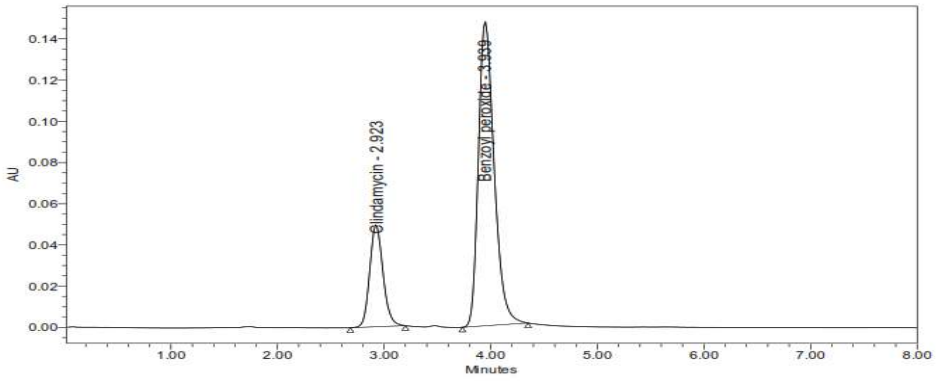

Figure 3: Optimized method chromatogram for Clindamycin and Benzyl peroxide

System Suitability: Tailing factor for the peaks due to Clindamycin and Benzoyl phosphate in Standard solution should not be more than 2.0, Theoretical plates for the Clindamycin and Benzoyl phosphate peaks in Standard solution should not be less than 2000

S.No	Name	RT(min)	Area (µVsec)	Height(µV)	Resolution	USP tailing	USP Plate count
1	Clindamycin	2.923	5487	298	5.2	1.02	3645
2.	Benzoyl phosphate	3.939	32145	4754		0.24	5428

Table 1: Results of system suitability parameters

Validation Parameters:

Assay:

S.No	Solution	Drug	RT (min)	Area (µ Vsec)	Height (µV)	Resolution	USP tailing	USP plate count
1	Standard	Clindamycin phosphate	2.923	5531	13536	5.4	1.52	2080
		Benzoyl peroxide	3.939	35983	18461		1.08	5023
2	Sample	Clindamycin phosphate	2.919	5485	13568	5.3	1.45	3096
		Benzoyl peroxide	3.939	35521	18483		1.09	4862

Table 2: Chromatogram for standard and Sample

Linearity:

S.no.	Clindamycin phosphate		Benzyl peroxide	
	Concentration (µg/ml)	Peak Area	Concentration (µg/ml)	Peak Area
1	4	1826	20	10765
2	8	3648	40	22485
3	12	5372	60	33169
4	16	7392	80	42863
5	20	9175	100	53548

Table 3 : Area of different concentration of clindamycin phosphate and benzyl peroxide

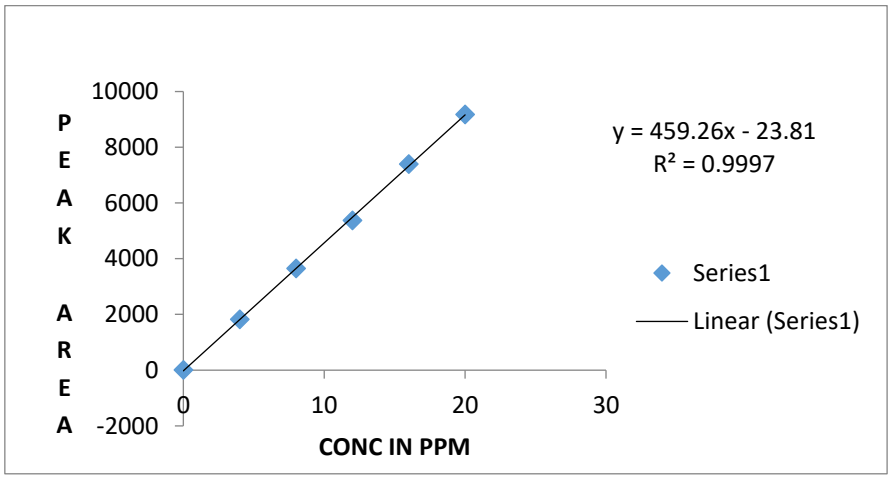

Figure 4: Calibration graph for Clindamycin phosphate

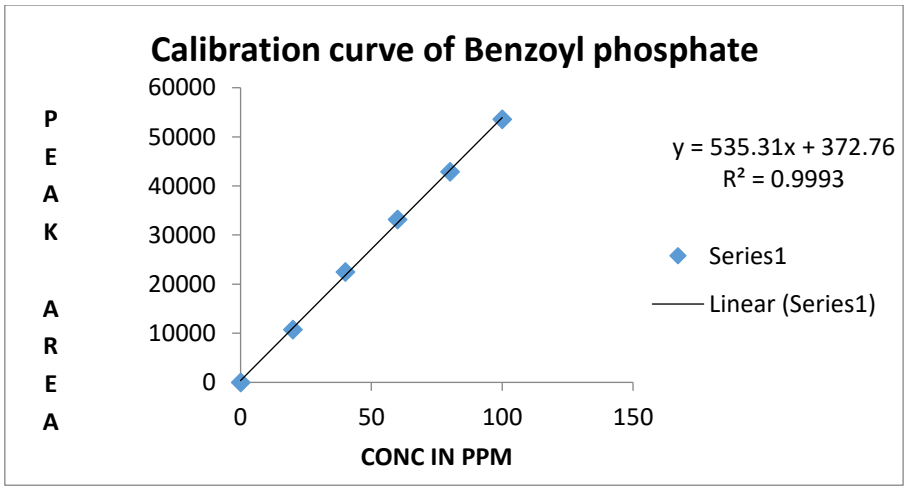

Figure 5: Calibration graph for Benzyl peroxide

Precision:

Injection	Clindamycin phosphate Peak Area	Benzyl peroxide peak Area
Injection-1	5493	34063
Injection-2	5445	34742
Injection-3	5458	34523
Injection-4	5466	34450
Injection-5	5420	34012
Injection-6	5414	34167
Average	5449.333	34326.17
Standard Deviation	29.62206	289.9265
%RSD	0.5	0.8

Table 4 : Results of Precision for Clindamycin phosphate and Benzyl peroxide

Intermediate precision:

Injection	Clindamycin phosphate Peak Area	Benzyl peroxide Peak Area
Injection-1	5522	35301
Injection-2	5547	35053
Injection-3	5569	35682
Injection-4	5535	35123
Injection-5	5525	35965
Injection-6	5530	35463
Average	5538	35431.17
Standard Deviation	17.54993	347.5137
%RSD	0.3	1.0

Table 5 : Results of Intermediate precision for Clindamycin phosphate and Benzyl peroxide

Accuracy:

Drug	% Conc (at specification Level)	Peak area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
Clindamycin phosphate	50%	2745	2	1.98	99.1	99.4
	100%	5531	4	3.6	99.0	
	150%	8328	6	6.0	100.2	
Benzyl peroxide	50%	18082	10	10.0	100.3	99.7
	100%	35752	20	19.8	99.2	
	150%	53836	30	29.9	99.5	

Table 6 : Accuracy (recovery) data for Clindamycin phosphate and Benzyl peroxide

LOD & LOQ:

Drug name	Baseline noise (µ V)		Signal obtained (µV)		S/N		Concentration	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
Clindamycin phosphate	68	68	197	662	2.90	9.74	0.50 µg/ml	0.84 µg/ml
Benzyl peroxide	68	68	200	674	2.94	9.91		

Table 7 : The results of LOD & LOQ of Clindamycin phosphate & Benzyl peroxide

CONCLUSION

The developed RP-HPLC method was validated as per ICH guidelines and proved to be precise, accurate, sensitive, and robust for the simultaneous estimation of Clindamycin phosphate and Benzoyl peroxide. The %RSD values for precision and intermediate precision were within acceptable limits, confirming reproducibility. Recovery studies indicated high accuracy with mean recoveries close to 100%. The LOD and LOQ values demonstrated the sensitivity of the method, while robustness studies confirmed its reliability under slight variations in chromatographic conditions. Therefore, the proposed method can be effectively applied for routine quality control analysis of these drugs in bulk and pharmaceutical formulations.

REFERENCES

1. United States Food and Drug Administration (FDA). Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics. Silver Spring: FDA; 2015.
2. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed. New Delhi: CBS Publishers; 2002.
3. Christian GD. Analytical Chemistry. 6th ed. New York: John Wiley & Sons; 2004.
4. Meyer VR. Practical High-Performance Liquid Chromatography. 5th ed. Chichester: John Wiley & Sons; 2010.

5. Kazakevich Y, LoBrutto R. HPLC for Pharmaceutical Scientists. Hoboken: John Wiley & Sons; 2007.
6. Chatwal GR, Anand SK. Instrumental Methods of Chemical Analysis. 5th ed. Mumbai: Himalaya Publishing House; 2007.
7. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. 3rd ed. New York: John Wiley & Sons; 2010.
8. Mandava vb, sudha bs, prasad kb. development and validation of a stability indicating reverse phase-high performance liquid chromatography method for simultaneous determination of clindamycin, metronidazole, and clotrimazole in pharmaceutical combined dosage forms development. 2017;10(1).
9. PubChem. Clindamycin; CID: 444446. National Center for Biotechnology Information, U.S.National Library of Medicine [Internet]. Available from: https://pubchem.ncbi.nlm.nih.gov
10. Barazandehtehrani m, namadchian m, fadayevatan s, souri e. derivative spectrophotometric method for simultaneous determination of clindamycin phosphate and tretinoin in pharmaceutical dosage forms. daru journal of pharmaceutical sciences. 2013 dec; 21:1-7.

HOW TO CITE: M. Venkataramana*, P. Sri Anjali, D. Shivaparameshwari, M. Navya Sri, M. Sri Vidhya, Daraksha Anjum, Development And Validation Of RP HPLC Method For The Simultaneous Estimation Of Clindamycin Phosphate And Benzoyl Peroxide In Pure And Dosage Forms, Int. J. Sci. R. Tech., 2026, 3 (6), 1477-1483. https://doi.org/10.5281/zenodo.20845867