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Stability Indicating HPLC Method For Simultaneous Estimation of Clindamycin Phosphate and Benzoyl Peroxide In Gel Formulation

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ABSTRACT

A simple, specific, accurate and stability-indicating reversed phase High Performance Liquid Chromatographic method was developed for the simultaneous determination of Clindamycin Phosphate and Benzoyl Peroxide, using a C_{18} column and a mobile phase composed of 20 mM Ammonium acetate buffer pH 4.0: Methanol (45: 55 %v/v) as mobile phase at flow rate of 1.2 ml/min with detection wavelength of 210nm. Retention times in RP-HPLC method were found to be 4.49 min, 8.78 min for Clindamycin Phosphate and Benzoyl Peroxide, respectively. Linearity of Clindamycin Phosphate and Benzoyl Peroxide were found in the range of 10.0-30.0 µg/ml and 25.0-75.1 µg/ml. The % recovery of Clindamycin Phosphate was found to be 98.45- 101.0 and 99.8- 99.38 for Benzoyl Peroxide. Both the drugs were subjected to acid, alkali, oxidation, thermal and sunlight degradation. The degradation products of Clindamycin Phosphate and Benzoyl Peroxide were well resolved from the pure drugs with significant differences in the retention time values. This method can be successfully employed for simultaneous quantitative analysis of Clindamycin Phosphate and Benzoyl Peroxide in gel formulation. The literature survey reveals that currently there is no stability indicating method has been reported for combination of Clindamycin Phosphate and Benzoyl Peroxide till date, Which can be applicable for routine analysis of combined formulation of drugs in quality control laboratories.

Keywords: Clindamycin Phosphate, Benzoyl Peroxide, degradation products, stability-indicating, HPLC

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INTRODUCTION

Chemical name of Clindamycin Phosphate (CLP) is (2S, 4R)-N-{2-Chloro-1-[(2R, 3R, 4S, 5R,6R)-3,4-dihydroxy-6-(methylsulfanyl)-5-(phosphonooxy)oxan-2-yl]propyly-1-methyl-4-propylpyrrolidine-2-carboximidic acid [1] (Figure- 1). Clindamycin (Phosphate) is antibacterial category. The mechanism of action of Clindamycin (Phosphate) has a primarily bacteriostatic effect. It is a bacterial protein synthesis inhibitor by inhibiting ribosomal translocation, in a similar way to macrolides. It does so by binding to the 50S rRNA of the large bacterial ribosome subunit. Clindamycin (phosphate) is an antibiotic useful for the treatment of a number of bacterial infections.[2] CLP is official in U.S.P and B.P.[3,4] .

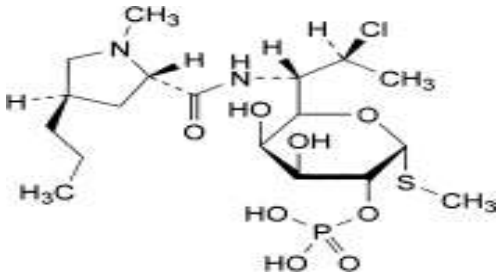

Figure 1: Chemical Structure of CLP

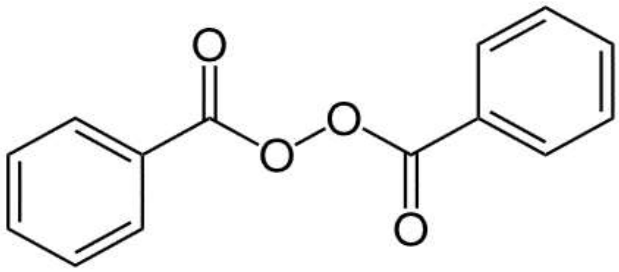

Figure 2: Chemical Structure of BNZ

Chemical name of Chemical name of Benzoyl peroxide (BNZ) is Dibenzoyl peroxide, Benzoperoxide [5] (Figure- 2). BNZ is antiseptic and antibacterial category. The anti bacterial action of BNZ is probably related to its ability, once in the skin, to release free radical oxygen. The drug is lipophilic; it penetrates the stratum corneum and enters the pilosebaceous follicle. It is rapidly broken down to benzoic acid and hydrogen peroxide and generates free radicals that oxidise proteins in bacterial cell membranes, exerting a bactericidal action. BNZ is used as an acne treatment, for bleaching flour, hair and teeth, for cross-linking polyester resins [6]. BNZ is official in B.P.[7]

Very few analytical methods have been reported for estimation of CLP and BNZ as a single ingredient as well as their combination with other drugs[8-10].

Central Drug Standard Control Organization **(**CDSCO) has approved Clindamycin Phosphate 1% and Benzoyl Peroxide 2.5%. Gel on 30th August 2011 in for the topical treatment of inflammatory vulgaris.

MATERIALS AND METHOD

Instrumentation

Weighing Balance used Scale Tec, Micro Balance - Mattler Toledo, pH meter - LAB INDIA, Melting point apparatus - LAB INDIA MR-VIS, IR AFFINITY-1 - SHIMADZU, UV Spectrophotometer - SHIMADZU, Sonicator - Sonorax, HPLC - Analytical technologies. Pump - P3000, Injector - I3000, Detector - UV3000, Column - Column, C_{18} (150X4.6) mm, 5µ were used.

Materials and reagents

Methanol - HPLC (Merck, India Limited), Ammonium acetate – AR grade (Merck, India Limited), Glacial acetic acid - Grade (Merck, India Limited), Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide (30%) Analytical Reagent (Merck, India Limited) were used.

Preparation of solutions

Preparation of 1N HCl

A solution of 1N HCl was prepared by taking 8.5 ml concentrated HCl in 100 ml volumetric flask and diluted up to mark with water.

Preparation of 1 N NaOH

A solution of 1N NaOH was prepared by dissolving 4 gm NaOH pallets in 100 ml water.

Preparation of 5% H_2O_2

A solution of 5 % H_2O_2 was prepared by taking 16.7 ml of 30 % H2O2 in 100 ml volumetric flask and diluted up to mark with water.

Preparation of mobile phase

Mobile phase was prepared by mixing 20 mM of Ammonium acetate buffer pH4.0: Methanol (45:55 %v/v).

Sample stock preparation: (Marketed formulation preparation)

An accurately weighed 2.0 g of gel was transferred into 100 ml volumetric flask carefully, added about 70ml of diluent in to it, shake for 15 minutes by mechanical means and further sonicate for 15 minutes with intermittent shaking, cool to attain room temperature and made up to volume with diluent and mixed well. Filter through 0.45µ syringe filter.

Standard preparation (20 µg/ml and 50 µg/ml CLP and BNZ respectively):

An accurately weighed 20mg of CLP and 50mg of BNZ were then transferred in 100ml of volumetric flask, dissolve and volume make up with diluent. Further dilute 5ml of this solution was transferred in to a 50ml volumetric flask and the volume was adjusted up to mark with diluent to get a concentration of CLP 20 µg/ml and BNZ 50 µg/ml.

Preparation of sample solutions

An accurately weighed 2 g of gel was transferred into 100 ml volumetric flask carefully, added about 70ml of diluent in to it, shake for 15 minutes by mechanical means and further sonication for 15 minutes with intermittent shaking, cool to attain room temperature and made up to volume with diluent and mixed well. Filter through 0.45µ syringe filter and further dilute 5ml of filtrate to 50ml with diluent to get a concentration of CLP 20 µg/ml and BNZ 50 µg/ml.

Forced degradation studies [11]

Forced degradation studies like acid/base, thermal, oxidation and sunlight of both the drugs were carried out. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the methods. The proposed stability – indicating RP-HPLC method was validated as per ICH Q1A (R2) guidelines. [12]

For acid degradation 5ml of 0.1N HCl for 24 hr the appropriate peak was not obtained. Then, 1N HCl (12 hours) 11.4% CLP degradation and 12.5% BNZ degradation were observed.

Base degradation with 5ml of 0.1N NaOH 24 hr the appropriate peak was not obtained. Then, treating with 1N NaOH (12 hours 14.6% CLP degradation and 17.2 % BNZ degradation were observed.

During oxidative degradation 5ml of 3% H_2O_2 for 24 hr the appropriate peak was not obtained. While treatment with 5 % H_2O_2 at room Temperature 19.2% CLP degradation and 11.3% BNZ degradation were observed.

Thermal degradation at 60°C for 12 hrs the appropriate peak was not obtained. Then, 15.3%. CLP degradation and 12.5% BNZ degradation were observed when samples were kept at 80 °C for 6 hrs.

12.6% CLP degradation and 14.3% BNZ degradation were observed in Sunlight degradation (12 hours).

METHOD VALIDATION

The proposed method has been developed and validated for the determination of CLP and BNZ in gel formulation. According to International Conference on Harmonization ICH Q2 (R1) guidelines [13], validation of the method was carried out by using linearity ,accuracy.

Linearity of the method was determined by making several dilutions of CLP and BNZ in mobile phase.

Accuracy is the closeness between the measured value and true values. Accuracy was determined by replicate analysis of samples containing known amount of analyte.

RESULTS AND DISCUSSION

Method development

By using different mobile phase ratio and flow rate were investigated. The optimum flow rate for which the column plate number (N) was maximum, with the best resolution between all components and with short run time was selected. (Table : 1)

Table 1: Final Optimized Chromatographic conditions:

Method parameter	Optimized Conditions
Mobile phase	20mM Ammonium acetate buffer pH 4.0 :Methanol (45:55 %v/v)
Column	Phenomanax Luna C_{18} (150X4.6)mm, 5µ
Column temperature	Ambient (25°C)
Injection volume	20µl
Flow rate	1.2 ml/min
Wavelength	210 nm
Diluent	Water: Methanol (50:50 %v/v)

Force degradation study

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acidic, alkaline, oxidative, thermal and sunlight degradations (Table-2).

Table 2: Results of forced degradation study of CLP and BNZ

Stress type	Time (hr)	% Assay		% Degradation	
		CLP	BNZ	CLP	BNZ
Control sample	As such sample	99.9%	100.0%	NA	NA
Acid , 5ml ,1N HCl	12	88.5%	87.5%	11.4%	12.5%
Base, 5ml, 1N NaOH	12	85.3%	82.8%	14.6%	17.2%
Oxidative, 5ml 5% H_2O_2	12	80.7%	88.7%	19.2%	11.3%
Thermal, At 80°C	6	84.6%	87.5%	15.3%	12.5%
Sunlight	12	87.3%	85.7%	12.6%	14.3%

Acidic degradation

5ml sample stock solutions of CLP and BNZ were taken into 50ml of volumetric flask, added 5ml 1N HCl into it and it was kept at room temperature for 12 hours. Then added 5ml of 1N NaOH to neutralize it and volume was made up to mark with diluent and mixed well and injected. Chromatogram is shown in (Figure -3,4).

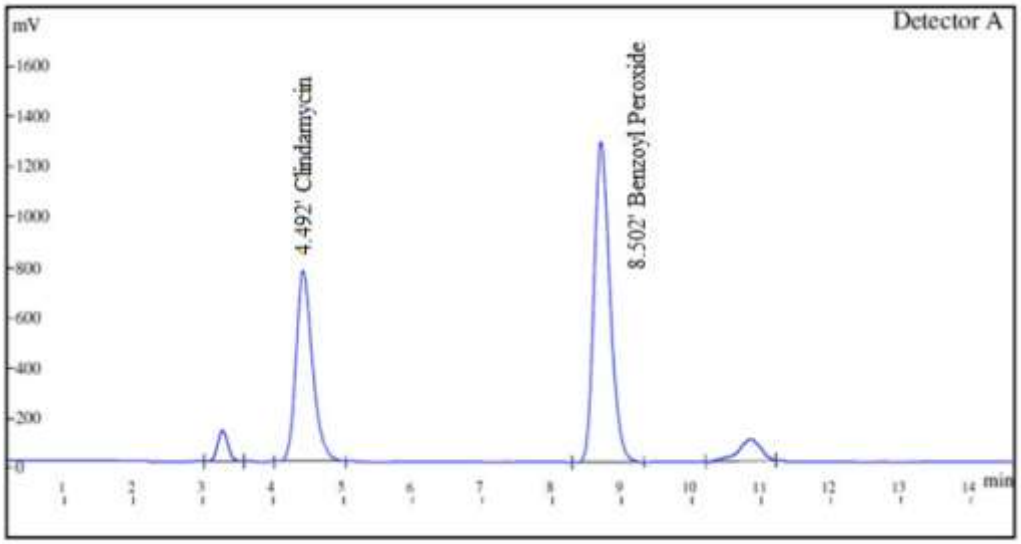

Figure 3: Chromatogram of Standard CLP and BNZ in acid degradation (1N HCl, 5 ml for 12 hour).

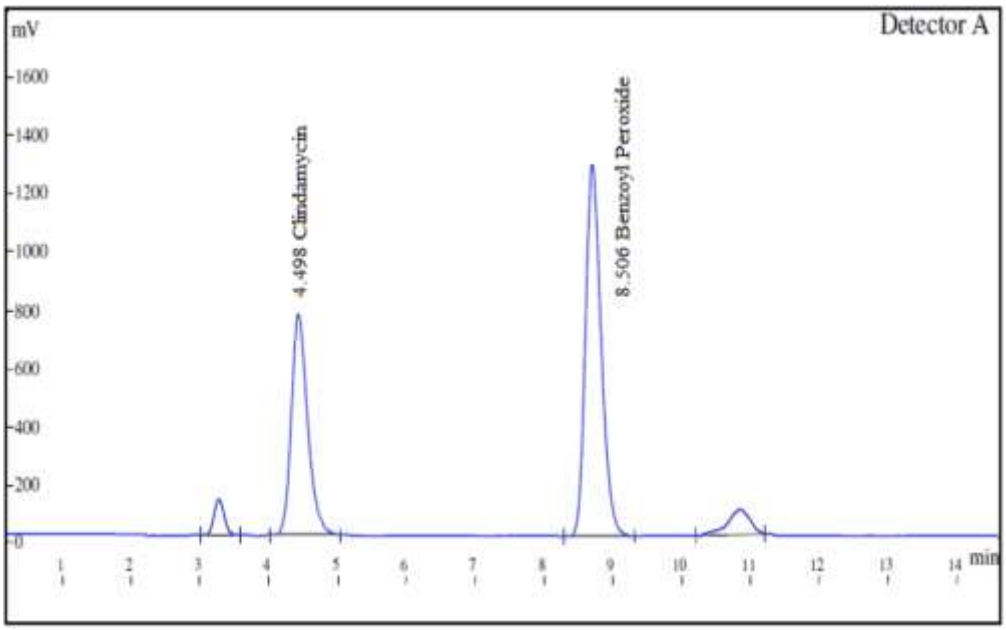

Figure 4: Chromatogram of sample CLP and BNZ in acid degradation (1N HCl, 5 ml for 12 hour).

Basic degradation

5ml sample stock solutions of CLP and BNZ were taken into 50ml of volumetric flask, added 5ml 1N NaOH into it and it was kept at room temperature for 12 hours. Then added 5ml of 1N HCl to neutralize it and volume was made up to mark with diluent and mixed well and injected. Chromatogram is shown in (Figure 5).

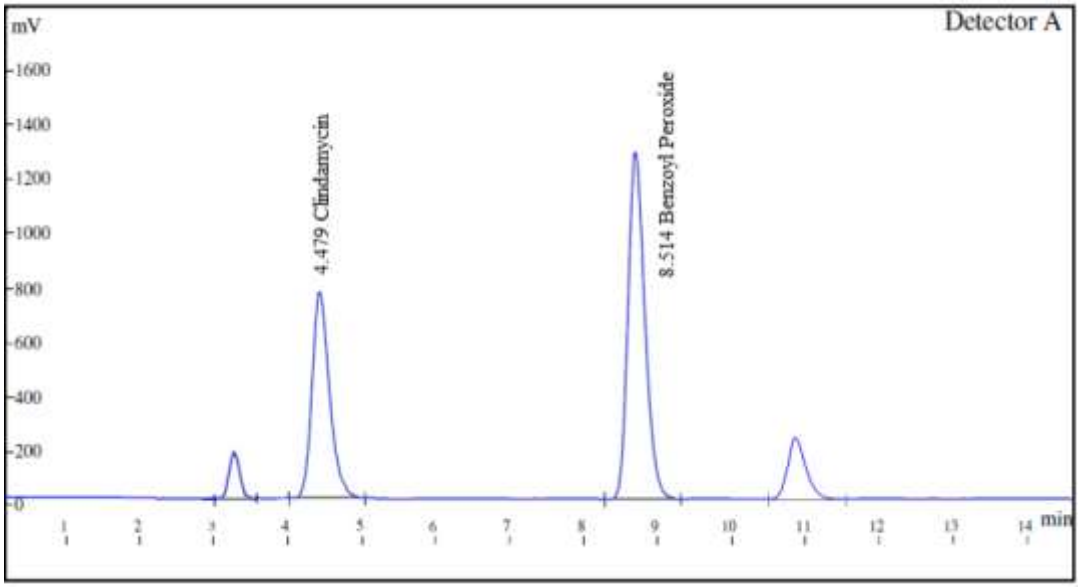

Figure 5: Chromatogram of CLP and BNZ in basic degradation (1N NaOH, 5ml for 12 hours).

Oxidative degradation

5ml sample stock solutions of CLP and BNZ were taken into 50ml of volumetric flask, added 5ml 5% H_2O_2 solution into it and it was kept at room temperature for 12 hours. Then volume was made up to mark with diluent and mixed well and injected. Chromatogram is shown in (Figure 6).

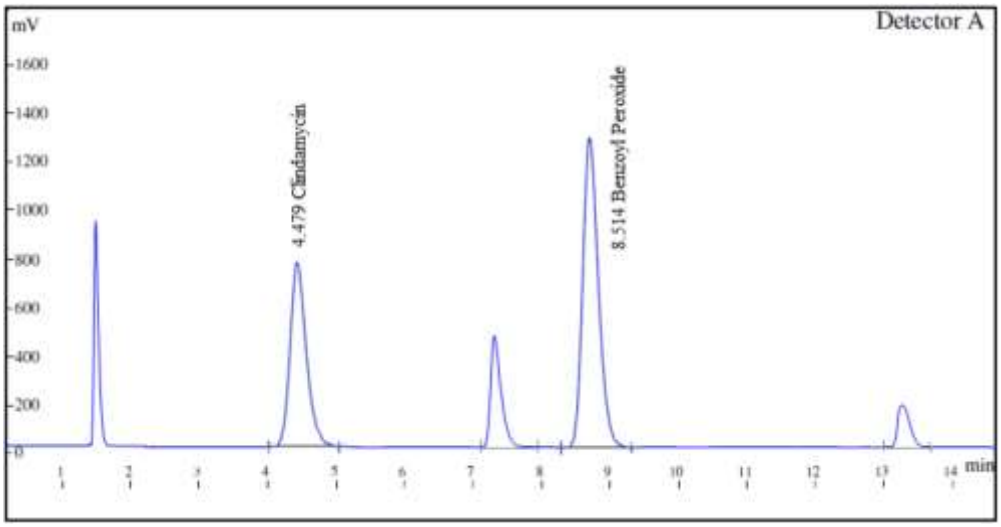

Figure 6: Chromatogram of CLP and BNZ in oxidative degradation (5 ml 5% H_2O_2 at Room Temp. for 12 hours).

Thermal degradation

5ml sample stock solutions of CLP and BNZ were taken into 50ml of volumetric flask; it was kept for 6 hours at 80°C temperature. Then volume was made up to mark with diluent and mixed well and injected.

Sunlight degradation

5ml sample stock solutions of CLP and BNZ were taken into 50ml of volumetric flask; it was kept for 12 hours in sunlight. Then volume was made up to mark with diluent and mixed well and injected.

METHOD VALIDATION

Linearity

The calibration curve was plotted between peak areas versus known concentrations of CLP and BNZ. The method shows linearity over a concentration range of 10.0 µg/ml to 30 µg/ml, the correlation coefficient was found to be 1.000 (Table- 3). For BNZ 25.0 µg/ml to 75.1 µg/ml, the correlation coefficient was found to be 1.000 (Table- 3,4).

Table 3: Calibration curve data for CLP

Linearity % level	Linearity for CLP		
	% Linearity Level	Conc. in µg/ml	Mean Area (n=3)
1	50	10.0	1959232
2	70	14.0	2746992
3	100	20.0	3926536
4	130	26.0	5103041
5	150	30.0	5915453
Correlation coefficient	1.000		
Regression Equation	197421x – 18169		
LOD (µg/ml)	0.32		
LOQ (µg/ml)	0.98		

Table: 4 Calibration curve data for BNZ

Linearity % level	Linearity for BNZ		
	% Linearity Level	Conc.in µg/ml	Mean Area (n=3)
1	50	25.0	4107203
2	70	35.0	5754848
3	100	50.1	8229748
4	130	65.1	10692874
5	150	75.1	12379258
Correlation coefficient	1.000		
Regression Equation	165222x – 28381		
LOD (µg/ml)	0.72		
LOQ (µg/ml)	2.19		

Accuracy

Accuracy was determined by replicate analysis of samples containing known amount of 80 - 120 % of the target amount (Table 5). The percentage recovery was 98.45 – 101.0 % for CLP and 99.8 – 99.38 % for BNZ accordingly (Table- 5).

Table 5: % Recovery Data of CLP and BNZ

Drug	% Level	Amount of sample taken (µg/ml)	Amount of standard spiked (µg/ml)	Total conc. (µg/ml)	Amount recovered	% Recovery
	80	10	8.09	18.09	15.93	98.45
CLP	100	10	10.22	20.22	20.59	100.6
	120	10	12.19	22.19	24.63	101.0
	80	25	20.05	45.05	40.03	99.8
BNZ	100	25	25.07	50.07	50.06	99.8
	120	25	30.08	55.08	59.79	99.38

CONCLUSION

An attempt has been made to develop simple, rapid, accurate methods for simultaneous estimation of Clindamycin Phosphate and Benzoyl Peroxide in gel formulation by High Performance Liquid Chromatography. This method was validated as per ICH guideline (Q2R1). For RP-HPLC linearity of Clindamycin Phosphate and BNZ were found in the range 10.0-30.0 µg/ml and 25.0-75.1 µg/ml. The result of the analysis of pharmaceutical formulation by the proposed method is reproducible and reliable. It is good agreement with the label claim of the drug. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the methods. The proposed stability – indicating RP-HPLC method was validated as per ICH Q1A (R2) guidelines. Stability indicating method can be successfully applied to perform long – term and accelerated stability studies in gel formulation. It can also be used to check quality of product after different storage condition and when stress degradation is carried out. The results indicated the suitability of the method to study stability of Clindamycin Phosphate and Benzoyl Peroxide under various forced degradation condition viz. acid, base, oxidative, thermal, sunlight degradation. It can be concluded that the developed method may be employed for analysis of stability samples of Clindamycin Phosphate and Benzoyl Peroxide since the method could separate the drugs from their degradation products.

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