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Original Research Paper

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CLINDAMYCIN PHOSPHATE AND BENZOYL PEROXIDE IN GEL FORMULATION

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ABSTRACT

A simple, rapid and sensitive Reverse Phase High Performance Liquid Chromatography method was developed for simultaneous estimation of Clindamycin Phosphate and Benzoyl Peroxide in gel formulation by using BDS Phenomanax Luna C_{18} (150X4.6) mm, 5µ and 20mM 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 for Clindamycin Phosphate and Benzoyl Peroxide were 4.49 min, 8.78 min respectively. The linearity of developed method was achieved in the range of 10.0-30.0 µg/ml for Clindamycin Phosphate and 25.0-75.1 µg/ml for Benzoyl Peroxide and limit of detection was found to be 0.32 µg/ml and 0.72 µg/ml and limit of quantification was found to be 0.98 µg/ml and 2.19 µg/ml for Clindamycin Phosphate and Benzoyl Peroxide respectively. The % Recovery of Clindamycin Phosphate was found to be 98.45 % -101.0% and 99.8 % -99.38 % for Benzoyl Peroxide. In a precision the repeatability % RSD was found to be 0.4 for Clindamycin Phosphate and 0.3 for Benzoyl Peroxide. Change in the ratio of mobile phase ±2.0 ml, Change in flow rate by ±0.2 ml/minute, Change in pH of mobile phase by ± 0.2 . Specificity of the method was ascertained by analysing standard drug and sample. No interfering peaks were found in the chromatogram by the proposed RP-HPLC method.

Keywords: Development, Validation, Clindamycin phosphate, Benzoyl peroxide, Gel formulation.

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-(phosphono-oxy)oxan-2-yl]propyly-1-methyl-4-propylpyrro-lidine-2-carbo- ximidic acid[1] (Figure-1). The category of CLP is antibacterial. The mechanism of action of Clindamycin (phosphate) has a primarily bacteriostatic effect CLP 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] 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 official in B.P.[6] 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.[7-10] 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[11], validation of the method was carried out by using accuracy, linearity, and precision, limit of detection, limit of quantitation, system suitability, and specificity. Robustness was

performed by deliberately changing the chromatographic conditions.

Central Drug Standard Control Organization (CDSCO) has approved CLP 1% and BNZ 2.5%. Gel on 30th August 2011 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, and 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 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.

Selection of Analytical Wavelength

Preparation of CLP: (10 µg/ml)

5mg of CLP dissolved in 50ml of methanol. Further dilute 5ml of this solution to 50ml with methanol.

Preparation of BNZ: (10 µg/ml)

5mg of BNZ dissolved in 50ml of methanol. Further dilute 5ml of this solution to 50ml with methanol.

Above standard solutions were scrutinized in the wavelength range of 200-400 nm using methanol as a blank in UV-Spectrophotometer (Figure-3,4).

Optimization of HPLC Method

It was found that mobile phase containing 20mM 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 for CLP and BNZ 4.49 min, 8.78 min (Figure-5), (Table-1).

Validation of the Method

Validation of the optimized RP-HPLC method was carried out with respect to the following parameters.

Linearity

The calibration curve was plotted between peak areas versus known concentrations of CLP. 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, For BNZ 25.0 µg/ml to 75.1 µg/ml, the correlation coefficient was found to be 1.000.

Sensitivity

The sensitivity measurement of CLP and BNZ by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated using following equations.

LOD = 3.3x σ/S

LOQ = 10x σ/S

Where,

σ = the standard deviation of the response

S = slope of the calibration curve.

Accuracy

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

Precision

The precision of the method was verified by repeatability, interday and intraday precision. Repeatability study was performed by analysis of three different concentrations of the drug in six replicates on the same day. Intraday precision was determined by analysing sample solutions at different time intervals on the same day and on different day for interday precision.

Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The important parameter to be studied was the resolution factor between two peaks. The robustness was checked by changing following parameters one by one:

Change in the ratio of mobile phase ±2.0 ml, Change in flow rate by ±0.2 ml/minute, Change in pH of mobile phase by ± 0.2 .

Specificity

Specificity of the method was ascertained by analysing standard drug and sample. No interfering peaks were found in the chromatogram by the proposed RP-HPLC method.

Quantitative Determination in Gel Formulation

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.

RESULTS AND DISCUSSION

The results of method development and validation studies on simultaneous estimation of CLP and BNZ in the current study involving 20 mM Ammonium acetate buffer pH 4.0: Methanol (45:55% v/v) as mobile phase for RP-HPLC are given below.

Method Development

CLP and BNZ were completely separated on C_{18} column by RP-HPLC using the isocratic elution of 20mM Ammonium acetate buffer and Methanol as mobile phase. When the methanol percentage was reduced starting from 80% by a decrement of every 5 %, broadening, fronting and tailing of peaks were observed. As a result of decrease in the percentage of methanol and using 20mM Ammonium acetate buffer pH 4.0 a sharp pointed and well separated peak was observed. As methanol concentration gradually decreases the peak broadening, fronting and tailing were remarkably reduced. 20mM Ammonium acetate buffer pH 4.0: Methanol (45:55% v/v) as mobile phase for RP- HPLC.

Linearity

The calibration curve was plotted between peak areas versus known concentrations of CLP . The method shows linearity over a concentration range of 10.0 µg/ml to 30 µg/ml. The correlation coefficient was found to be $r^2 = 1.000$ (Figure-6).

Linearity as well as the calibration curve BNZ in mobile phase in the range of 25.0- 75.1 µg/ml has been determined respectively (Figure-7).

Specificity

Specificity of the method was ascertained by analysing standard drug and sample. No interfering peaks were found in the chromatogram by the proposed RP-HPLC method (Figure- 8 and 9).

Accuracy

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

Precision

Precision describes the repeatability of analytical method. Six aliquots of each single concentration were prepared and analyzed on same day and three consecutive days. The developed method was found to be precise as the %RSD were < 2 %. Precision of the method was determined by intraday and inter-day studies. The different concentration of CLP 10.0 µg/ml - 30.0 µg/ml their RSD is less than 2 that is between 0.10 – 0.41 and BNZ different concentration is taken 25.0 µg/ml – 75.0 µg/ml their RSD is less than 2 that is between 0.35 – 0.86, Whereas inter-day different concentration of CLP 10.0 µg/ml - 30.0 µg/ml their RSD is less than 2 that is between 0.61 – 0.84 and BNZ different concentration is taken 25.0 µg/ml – 75.0 µg/ml their RSD is less than 2 that is between 0.66-1.04 .

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in the analytical procedure parameters [pH (±0.2), Flow rate (±0.2 ml) and proportion of mobile phase (±2.0 v/v]. The standard deviation of the peak is calculated for each parameter and the %RSD was found to be less than 2% (Table-2).

System Suitability

Various System Suitability parameters were calculated. The parameters were found within acceptance criteria (Table- 3).

Quantitative Determination in Gel Formulation

In a quantitative determination % assay of CLP was found to be 99.9% and BNZ was found to be 99.7% (Table-4).

Summary of all the validation parameter has been mention hear (Table-5).

CONCLUSION

Development and validation of RP-HPLC method was found to be simple, specific, robustness, accurate, precise and economical. These methods can be applied for routine quantitative analysis of Clindamycin Phosphate and Benzoyl Peroxide in gel formulation. 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.

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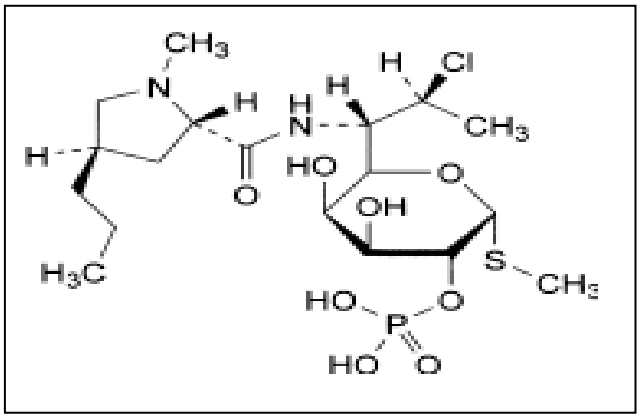

Figure 1: Chemical structure of CLP

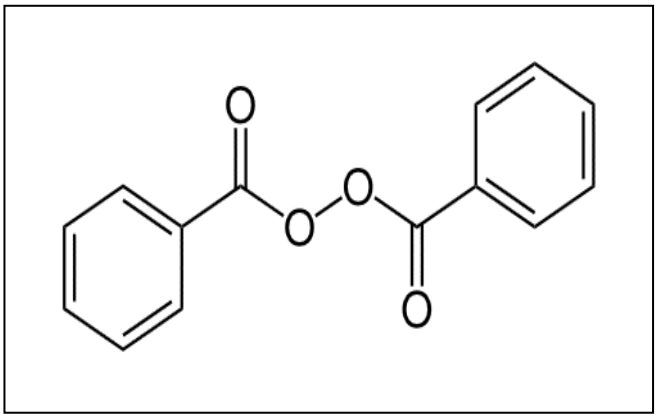

Figure 2: Chemical structure of BNZ

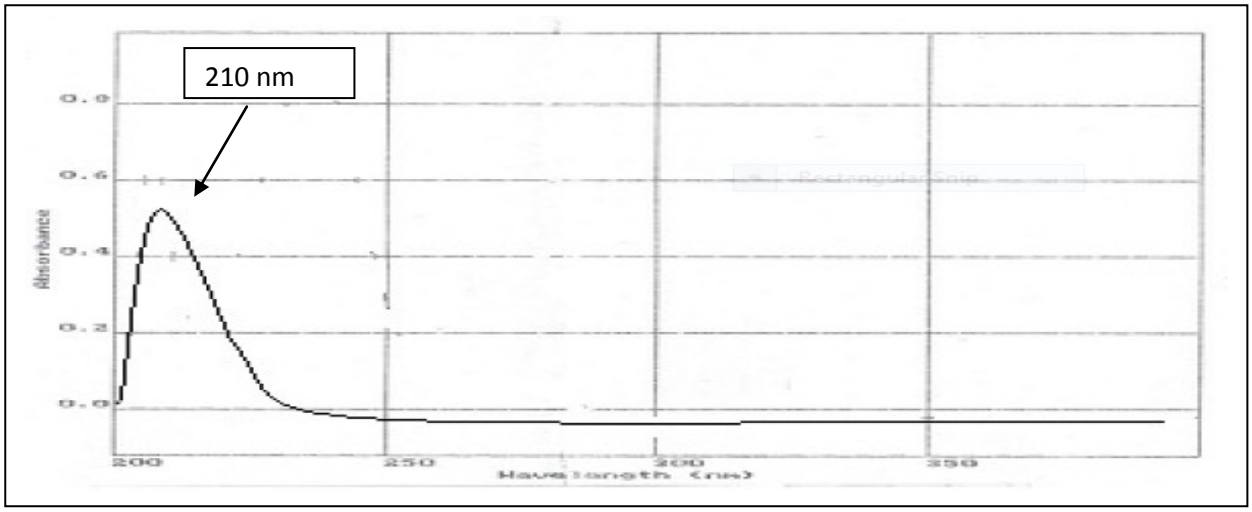

Figure 3: UV Spectrum of CLP

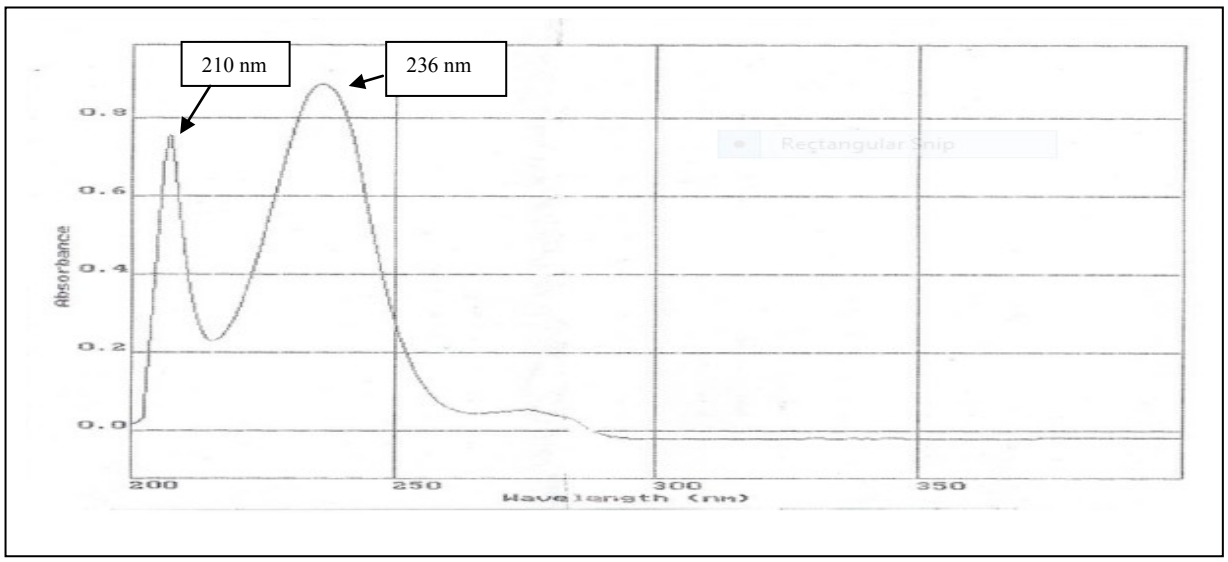

Figure 4: UV Spectrum of BNZ

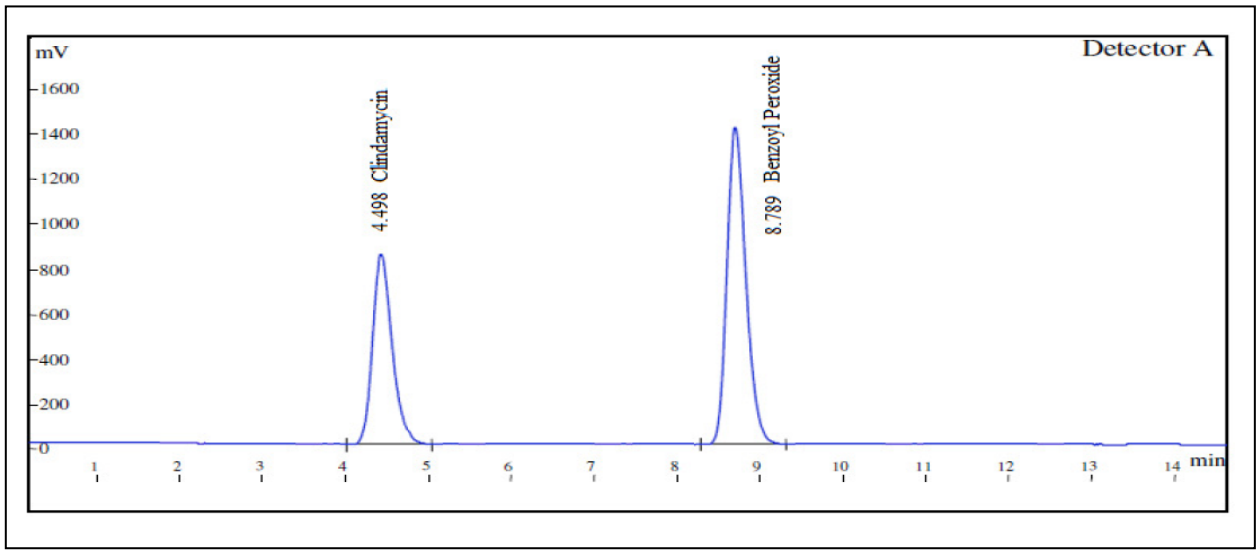

Figure 5: Chromatogram of standard mixture for CLP and BNZ (20 µg/ml and 50 µg/ml)

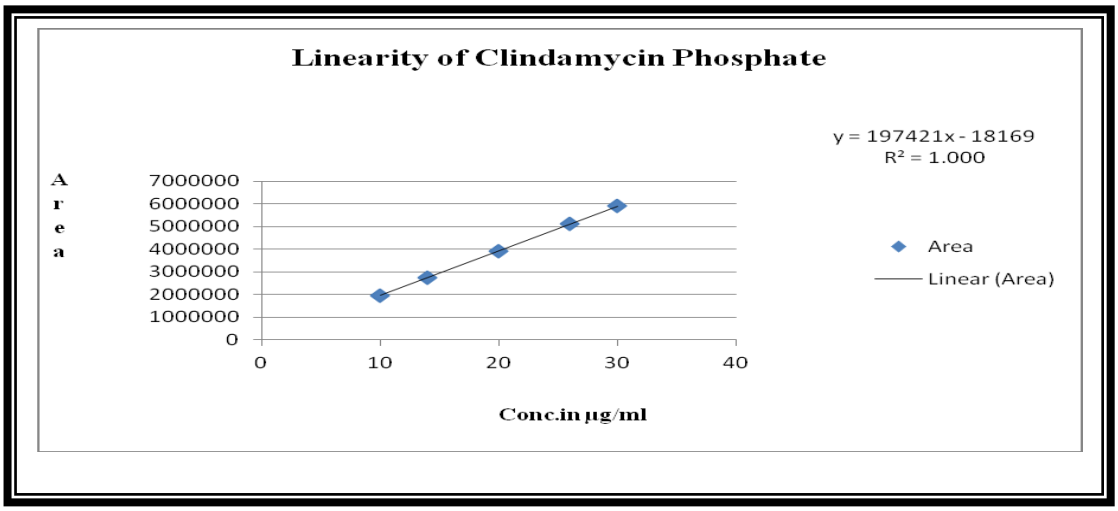

Figure 6: Calibration curve of CLP at 210 nm

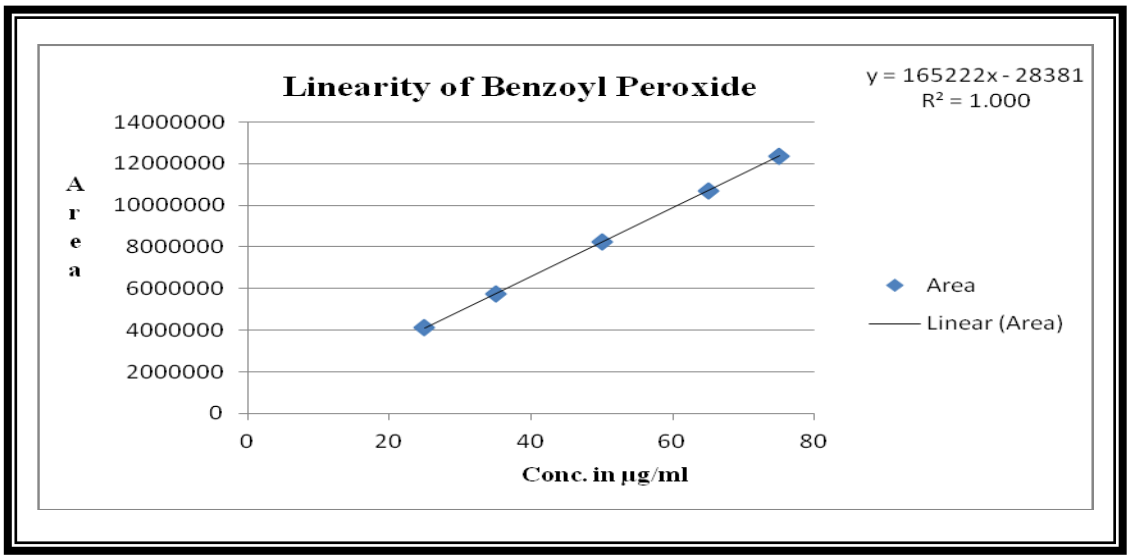

Figure 7: Calibration curve of BNZ at 210nm

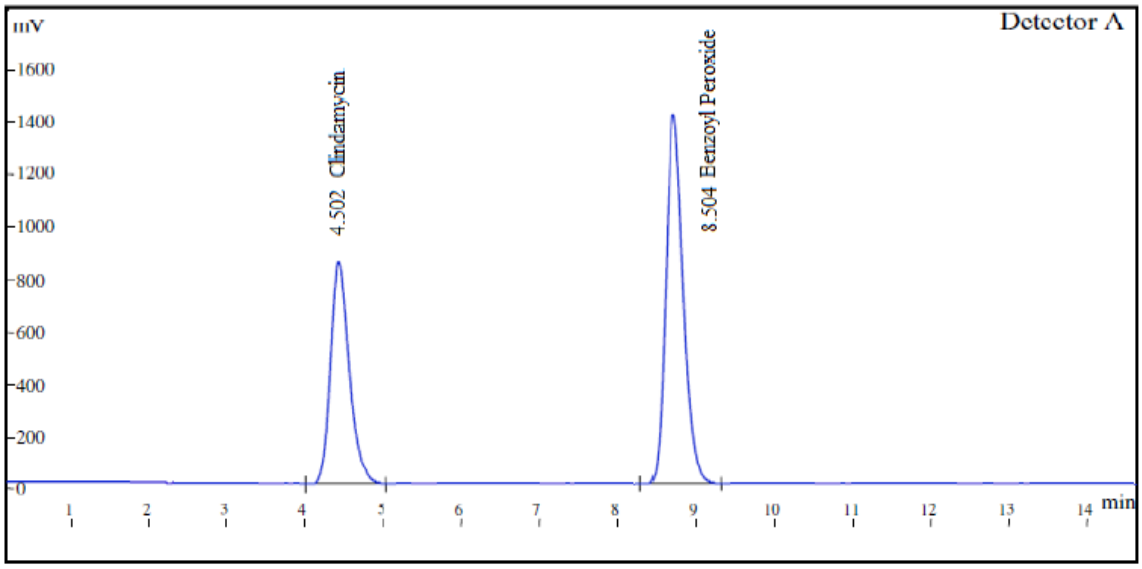

Figure 8: Chromatogram of standard CLP (20µg/ml) and BNZ (50 µg/ml) using mobile phase 20mM Ammonium acetate buffer pH 4.0: Methanol (45:55 %v/v).

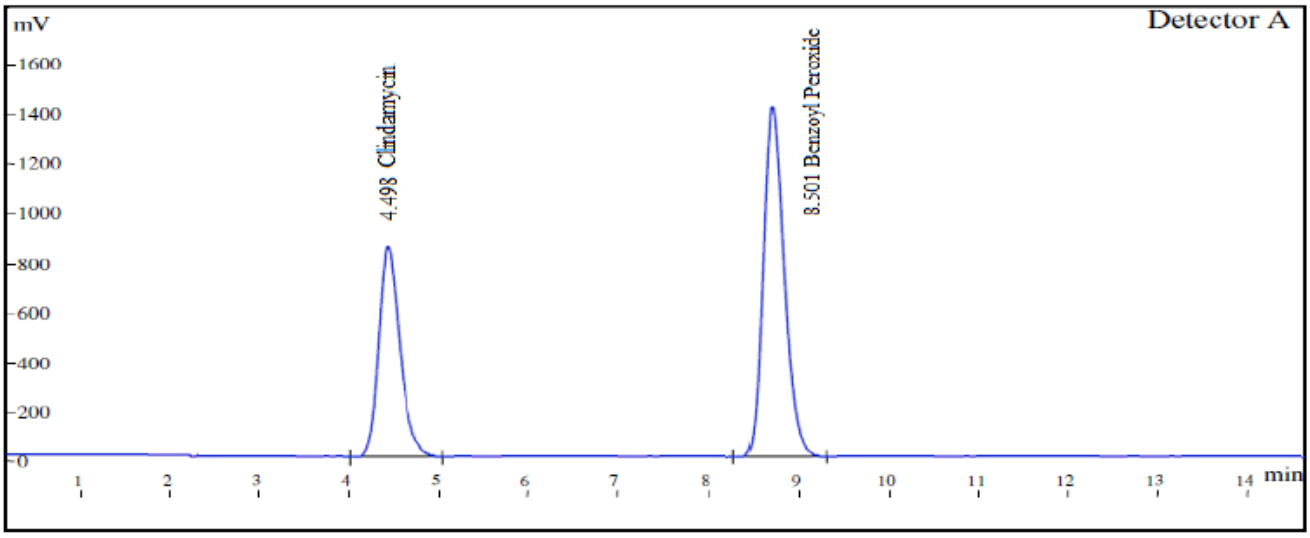

Figure 9: Chromatogram of CLP (20µg/ml) and BNZ (50 µg/ml) of gel formulation using mobile phase 20 mM Ammonium acetate buffer pH 4.0: Methanol (45:55 %v/v)

Table 1: Optimized Chromatographic conditions of CLP and BNZ

Parameters	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)

Table 2: Robustness study for CLP and BNZ

Factor	Level	CLP				BNZ			
		RT (n=6)	% RSD	Mean Area (n=6)	% RSD	RT (n=6)	% RSD	Mean Area (n=6)	% RSD
Flow Rate ml/min									
1.0	-0.2	4.959	0.3	4265705	0.7	9.373	0.3	8997394	0.7
1.2	0	4.501	0.0	3886713	0.4	8.504	0.1	8179449	0.3
1.4	+0.2	9.373	0.5	3530398	0.3	7.671	0.4	7375098	0.4
% Mobile phase %v/v									
43:57	-2.0	4.141	0.0	3902875	0.2	7.909	0.1	8192301	0.2
45:55	0	4.501	0.0	3886713	0.4	8.504	0.1	8179449	0.3
47:53	+2.0	5.056	0.7	3903941	1.0	9.442	0.3	8124886	0.5
pH of mobile phase									
3.8	-0.2	4.096	0.5	3891570	0.3	8.718	0.4	8192301	0.2
4.0	0	4.501	0.0	3886713	0.4	8.504	0.1	8179449	0.3
4.2	+0.2	4.910	0.2	3890666	0.4	8.853	0.1	8131745	0.4

Table 3: System Suitability Parameters

Parameters	CLP (n =6)	BNZ (n =6)	Acceptance Criteria
Theoretical Plates*	28315.16	31869.5	>2000
Retention time (min)*	4.501	8.505	-
Tailing factor*	1.1	1	<1.5
Resolution	-	10.18	>2.0

*Mean (n=6)

Table 4: Analysis of Marketed Formulation

Gel	Amount of Drug taken (µg/ml)		Amount found (µg/ml)		% Assay	
	CLP	BNZ	CLP	BNZ	CLP	BNZ
Acanya Gel	20.02	50.11	19.98	49.96	99.9	99.7
			20.03	49.82	100.0	99.4
			20.19	50.11	100.9	100.0
			% RSD		0.5	0.3

Table 5: Regression analysis data & summary of validation parameter for proposed method

Parameters	Results	
	CLP	BNZ
Linear Range (n=3) (µg/ml)	10.0-30.0	25.0-75.1
Slope	197421	165222
Intercept	18169	28381
Limit of Detection(µg/ml)	0.32	0.72
Limit of Quantification (µg/ml)	0.98	2.19
Regression equation	y = 197421x – 18169	y = 165222x – 28381
Correlation co-Efficient(r^2)	1.000	1.000
Assay recovery (%)	98.45-101.0	99.8-99.38
Repeatability (%RSD)	0.4	0.3
Intra-day (n=3)	0.10-0.41	0.35-0.86
Inter-day (n=3)	0.61-0.84	0.66-1.04
Specificity	Specific	Specific
Robustness	Robust	Robust

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