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A new RP-HPLC Method Development and Validation for the Erythromycin and Benzyl peroxide in Bulk and Tablet Dosage Form

Mahendar T*, G. Kalpana Devi

Department of Pharmaceutical Analysis & Quality Assurance, Sai Pranavi College of Pharmacy, Keesara, R.R dist-501301, Telangana, India

A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Erythromycin and Benzyl peroxide by using Agilent C_{18} Column (250mm x 4.6mm), flow rate was 1ml/min, mobile phase ratio was Methanol:Acetonitril (70:30 v/v), detection wavelength was 238 nm. The Spectroscopic method was done in solvent using methanol and the instrument lab India 3000+ with UV win software. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector, Empower-software version 2. The retention times were found to be 2.443 min and 2.918 min. The % purity of Erythromycin and Benzyl peroxide were found to be 100.7 and 101.4 respectively. The linearity study of Erythromycin and Benzyl peroxide were found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r^2) was found to be 0.999 and 0.999 respectively, % recovery for Erythromycin and Benzyl peroxide were found to be 100 and 100.5. %RSD for repeatability and precision was found to be <2. LOD values were 2.95 and 3.04 and LOQ value was 9.87 and 10 respectively for Erythromycin and Benzyl peroxide.

Keywords: Erythromycin, Benzyl peroxide, HPLC.

A R T I C L E I N F O

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***Corresponding Author**
Mahendar T
Dept. of Pharmaceutical Analysis & Quality Assurance, Sai Pranavi College of Pharmacy, Keesara, R.R dist-501301, Telangana, India
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1. Introduction

Erythromycin is a macrolide antibiotic produced by Streptomyces erythreus. It inhibits bacterial protein synthesis by binding to bacterial 50S ribosomal subunits; binding inhibits peptidyltransferase activity and interferes with translocation of amino acids during translation and assembly of proteins Erythromycin may be bacteriostatic or bactericidal depending on the organism and drug concentration [1, 2]. Benzoy peroxide (BPO**)** is an organic compound in the peroxide family. It consists of two benzoyl groups bridged by a peroxide link [3]. Its structural formula is $[C_6H_5C(O)]_2O_2$. It is one of the most important organic peroxides in terms of applications and the scale of its production [4]. Benzoyl peroxide is used as an acne treatment, for bleaching flour, hair and teeth, for cross-linking polyester resins, and many other uses [5].

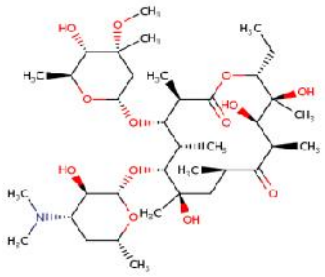

Figure 1: Erythromycin

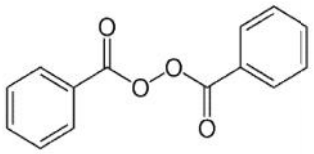

Figure 2: Benzyl peroxide

Analytical methods

The technique employed in qualitative and quantitative analysis is based upon the performance of suitable chemical reactions and either measuring the amount of reagent needed to complete the reaction, or ascertaining the amount of reaction product obtained [6]. Quality is important in every product or service but it is vital in medicine as it involves life. Unlike ordinary consumer goods there can be no "second quality" in drugs [7]. Quality control is a concept, which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production [8]. Physico-chemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions Among the Physico-chemical methods, the most important are optical (Refractometry, Polarimetry, Emission, Fluorescence [9], Photometry including photocolorimetry, Spectrophotometry covering UV-Visible and IR regions and Nephelometry or Turbidimetry) and chromatographic (Column, Paper, TLC, GLC, HPLC) methods [10]. Methods such as Nuclear Magnetic Resonance and Para Magnetic Resonance are becoming more and more popular [11]. The combination of Mass Spectroscopy with Gas Chromatography and Liquid Chromatography are the most powerful tools available [12].

The number of new drugs is constantly growing. This requires new methods for controlling their quality. Modern pharmaceutical analysis must need the following requirements [13].

- The analysis should take a minimal time.
- The accuracy of the analysis should meet the demands of pharmacopeia
- The analysis should be economical.
- The selected method should be precise and selective.

2. Materials and Methods

Apparatus

The instrument used for the study was Alliance Waters HPLC Auto Sampler, Separation module 2695, photo diode array detector with Empower-software version-2[14].

Reagents and Materials

The solvents used were Methanol, Acetonitril, Potassium dihydrogen ortho phosphate and HPLC Water [15].

Selection of chromatographic condition

Proper selection of the method depends upon the nature of the sample, its molecular weight and solubility [16]. The drugs selected in the present study are polar in nature and hence reversed phase or ion-pair or ion exchange chromatography method may be used [17]. The reversed phase HPLC was selected for the separation because of its simplicity and suitability [18].

Selection of detection wavelength:

10 mg of Erythromycin and Benzyl peroxide was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained [19]. The overlay spectrum was used for selection of wavelength for Erythromycin and Benzyl peroxide. The isobestic point was taken as detection wavelength [20].

Selection of mobile phase:

Methanol: ACN (70:30%v/v) has been selected as mobile phase. If any buffer selected buffer pH should be between 2 to 8. If the buffer pH is below 2 siloxane linkages are cleaved [21]. If the buffer pH is above 8 dissolution of silica takes place. pH controls the elution properties by controlling the ionization characteristics [22]. It also decreases the retention and improves separation [23]. Good Response, Area, Tailing factor, Resolution will be achieved

Chromatographic trials for Simultaneous Estimation of Erythromycin and Benzyl peroxide by RP- HPLC.

Parameters Description

Trial-1 Chromatographic conditions

Column : Inertsil C18 (4.6*150mm) 5µm
Mobile phase ratio : Water: Methanol (40:60%v/v)
Detection wavelength : 232 nm
Flow rate : 1ml/min
Injection volume : 10µl
Column temperature : Ambient
Auto sampler temperature : Ambient

Observation: Erythromycin and Benzyl peroxide were separated and two individual peaks are displayed. But they are not clear**.**

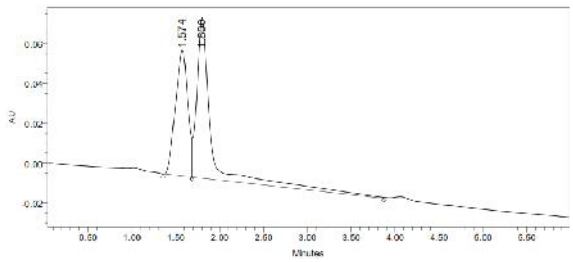

Figure 3: Chromatogram of Trial-1

Trial-2 Chromatographic condition

Parameters Description

Column : Inertsil C18 (4.6*150mm) 5µm
Mobile phase ratio : Water: Methanol (40:60%v/v)
Detection wavelength : 238nm
Flow rate : 1ml/min
Injection volume : 10µl
Column temperature : 40°
Auto sampler temperature: Ambient

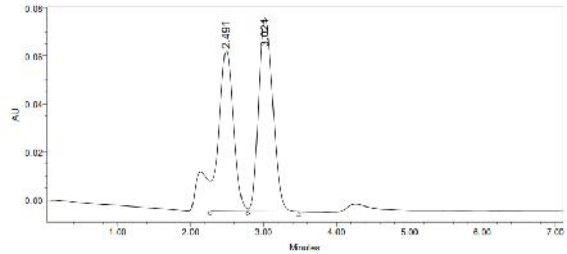

Figure 4: Chromatogram of Trial-2

Observation: Peak S symmetry is being improved when compared to the previous trial. Further trials are conducted for better resolution.

Trial-3 Chromatographic condition

Parameters Description

Column : Xterra C18 5µm (4.6*250mm)
Mobile phase ratio : Phosphate buffer pH 3.0: Methanol (50:50%v/v)
Detection wavelength : 232nm
Flow rate : 1ml/min
Injection volume : 10µl
Column temperature : Ambient Auto sampler

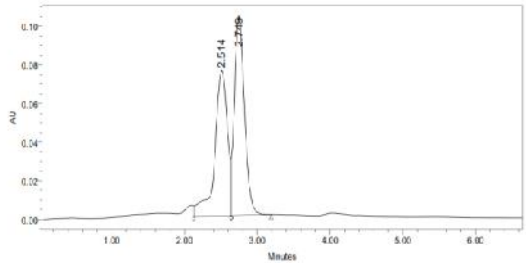

Figure 5: Chromatogram of Trial-3

Observation: There is noticeable improvement in resolution. But peak symmetry is not achieved.

Trial-4 Chromatographic condition

Parameters Description

Column : Agilent C18 5µm (4.6*250mm)
Mobile phase ratio: Phosphate buffer pH 3.0: Methanol (40:60%v/v)
Detection wavelength : 238nm
Flow rate : 1ml/min
Injection volume : 10µl
Column temperature : Ambient
Auto sampler temperature : Ambient

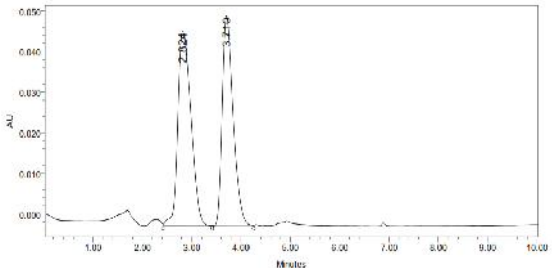

Figure 6: Chromatogram of Trial-4

Observation: Resolution and peak symmetry are upto the limit. Further trials are made for System suitability parameters to be within the limit.

Trial-5 Chromatographic condition (optimized method)

Parameters Description

Column : Agilent C18 5µm (4.6*250mm)
Mobile phase ratio : Methanol: ACN (70:30 %v/v)
Detection wavelength : 238nm
Flow rate : 1ml/min
Injection volume : 10µl
Column temperature : Ambient

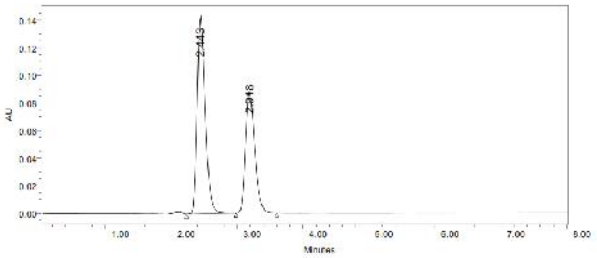

Figure 7: Chromatogram of Trial-5(optimized method)

Observation: The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence, this method is chosen as optimized one.

Procedure

Preparation of mobile phase:

A mixture of Methanol 700ml (70%), 300 mL of ACN (30%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration [25].

Standard Preparation for Erythromycin:

10mg of Erythromycin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluant [26].

Standard Preparation for Benzyl peroxide:

10mg of Benzyl peroxide working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent [27].

3. Results and Discussion

Method Validation Parameters

1. Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank [28].

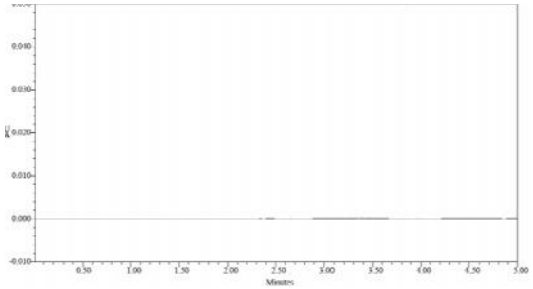

Figure 8: Chromatogram of Blank

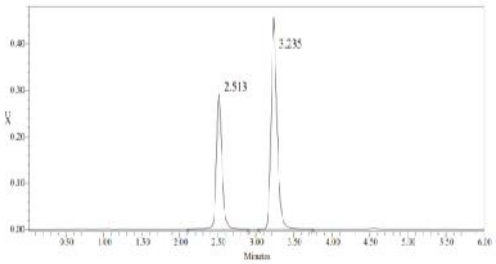

Figure 9: Chromatogram of Sample

2. Linearity

The linearity study was performed for the concentration of 100 ppm to 500 ppm and 1 ppm to 5 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient [29]

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range

The linearity study was performed for concentration range of 1µg-5µg and 100µg-500µg of Erythromycin and Benzyl peroxide and the correlation coefficient was found to be 0.999 and 0.999. (NLT 0.999).

4. Accuracy

The accuracy study was performed for 50%, 100% and 150 % for Erythromycin and Benzyl peroxide. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery [30].

Acceptance criteria:

Correlation coefficient should be not less than 0.999.

Assay procedure: The standard solution, Accuracy -50%, Accuracy -100% and Accuracy-150% solutions were injected [31]. The Amount found and Amount added for Benzyl peroxide & Erythromycin and the individual recovery and mean recovery values were calculated [32].

5. Precision

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported [33]. The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and % RSD were calculated and reported.

Acceptance criteria

The % RSD for the area of six sample injections results should not be more than 2.

Selection of solvent

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10 µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400 nm. The overlay spectrum of Erythromycin and Benzyl peroxide was obtained and the isobestic point of Erythromycin and Benzyl peroxide showed absorbance's maxima at 238 nm.

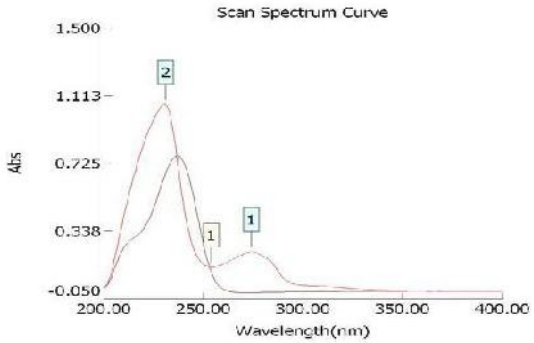

Figure 10: Overlain Spectra of Erythromycin and Benzyl peroxide

Validation of the method

Linearity: (Erythromycin and Benzyl peroxide)

The linearity study was performed for the concentration of 100 ppm to 500 ppm and 1 ppm to 5 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Calibration graph for Erythromycin and Benzyl peroxide are shown in below.

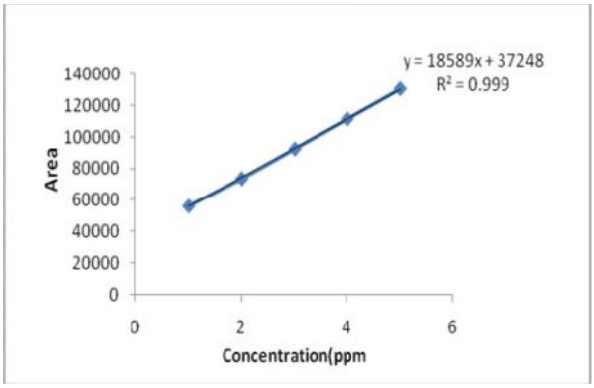

Figure 11: Calibration graph of Erythromycin

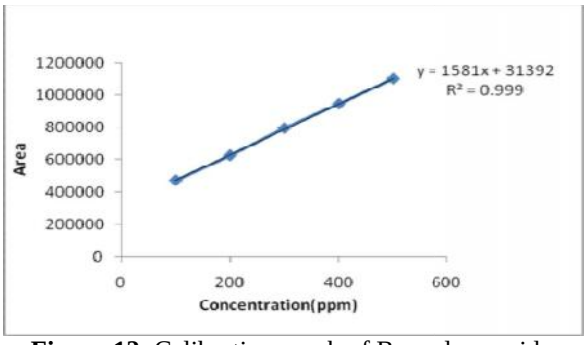

Figure 12: Calibration graph of Benzyl peroxide

Table 1: Calibration data for Erythromycin

S. No	Linearity Level	Concentration	Area
1	I	1ppm	56472
2	II	2ppm	73841
3	III	3ppm	92655
4	IV	4ppm	111541
5	V	5ppm	130567
Correlation Coefficient			**0.999**

Table 2: Calibration data for Benzyl peroxide

S. No	Linearity Level	Conc.	Area
1	I	100ppm	471603
2	II	200ppm	626230
3	III	300ppm	794999
4	IV	400ppm	946124
5	V	500ppm	1102139
Correlation Coefficient			**0.999**

Recovery studies

The accuracy study was performed for 50%, 100% and 150 % for Erythromycin and Benzyl peroxide. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery.

Detection limit:

The LOD was performed for Erythromycin and Benzyl peroxide was found to be 2.95 and 3.04 respectively.

Quantitation Limit

The LOQ was performed for Erythromycin and Benzyl peroxide was found to 9.87 and 10 respectively.

Table 3: Showing accuracy results for Erythromycin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	564367	5	5.0	101.1%	102.30%
100%	1115445	10	10.5	105.5%	
150%	1682465	15	15.0	100.5%	

Table 4: Showing accuracy results for Benzyl peroxide

%Concentration (at specification level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1829739	5	5.02	100.1%	100.19%
100%	3642243	10	9.96	99.60%	
150%	5535371	15	15.1	101.30%	

Table 5: Showing system suitability results for Erythromycin

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	**0.8**	**5688.6**	**1.3**
2	**1.0**	**5095.5**	**1.3**
3	**1.2**	**4449.8**	**1.3**

Table 6: Showing system suitability results for Benzyl peroxide

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	4227.9	1.4
2	1.0	3873.2	1.4
3	1.2	3288.7	1.3

Table 7: Repeatability results for Erythromycin

S.No	Peak name	RT	Area
1	Erythromycin	2.506	3367917
2	Erythromycin	2.516	3324161
3	Erythromycin	2.219	3390163

4	Erythromycin	2.531	3323428
5	Erythromycin	2.544	3329454
Mean			3347025
Std.dev			30354.9
%RSD			0.9

Table 8: Repeatability results for Benzyl peroxide

	Peak name	**RT**	**Area**
1	Benzyl peroxide	3.230	1025541
2	Benzyl peroxide	3.239	1023214
3	Benzyl peroxide	3.246	1023881
4	Benzyl peroxide	3.257	1020840
5	Benzyl peroxide	2.271	1026447
Mean			1023985
Std.dev			2178.2
%RSD			0.21

4. Conclusion

A new method was established for simultaneous estimation of Erythromycin and Benzyl peroxide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Erythromycin and Benzyl peroxide by using Agilent C18 5µm (4.6*250 mm) column, flow rate was 1 ml/min, mobile phase ratio was Methanol: ACN (70:30 %v/v), detection wave length was 238 nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 2.443 mins and 2.918 mins. The % purity of Erythromycin and Benzyl peroxide was found to be 100.7% and 101.4% respectively. The system suitability parameters for Erythromycin and Benzyl peroxide such as theoretical plates and tailing factor were found to be 1.3, 5117.5 and 1.4, 3877.3 the resolution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Erythromycin and Benzyl peroxide was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.6 and 0.16, % RSD for intermediate precision was 0.2 and 0.2 respectively. The precision study was precise, robust and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Erythromycin and Benzyl peroxide in API and Pharmaceutical dosage form.

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