

ANALYSIS OF ERYTHROMYCIN AND BENZOYL PEROXIDE IN COMBINED DOSAGE FORM BY UV-VISIBLE SPECTROPHOTOMETRY

ROHINI WANKHADE¹, SUHASINI BHALERAO¹, HITEN PANCHORY¹, ARUNA PUNDIR¹, RAM PRADHAN²

¹Department of Pharmaceutics, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, 421003, Maharashtra, India, ²Tureen Specialty Ingredients Pvt. Ltd., Pant Nagar, Ghatkopar, East Mumbai, Maharashtra - 400075, India

Received: 25 May 2012, Revised and Accepted: 11 July 2012

ABSTRACT

Gels containing a combination of Erythromycin and Benzoyl Peroxide are frequently used in the treatment of *Acne vulgaris*. A method was developed to determine the content of both Erythromycin and Benzoyl Peroxide in these gels. Erythromycin was analyzed from the gel by reacting with concentrated H₂SO₄. The detection wavelength was 480nm where benzoyl peroxide does not give any interference. Benzoyl Peroxide was detected at 236nm without any interference of Erythromycin since erythromycin has weak chromophore, on dilution erythromycin do not show peak in UV spectrophotometer. The specificity, repeatability, linearity and recovery of both methods were examined.

Keywords: Erythromycin, Benzoyl Peroxide, UV Visible analysis, Validation

INTRODUCTION

Acne is a common inflammatory disease in skin areas where sebaceous glands are largest, most numerous and active. In its milder type it is more or less superficial disorder which is evidenced by slight, spotty irritation. However, in more inflammatory types of acne, bacterial invasion of pilosebaceous follicles occurs and pustules, infected cysts and infected sacs appear. These lesions may become extensive and leave permanent, disfiguring scars¹.

Acne is characterized by pimples on the face. It affects individuals of all races covers 85% of teenagers, 42.5% of men, and 50.9% of women between the ages of 20 and 30 years. Spontaneous regression usually occurs after the age of 20, but some patients may continue suffering during adult life. In 2001, the global market for prescription acne products was estimated to be two billion dollars and the non-prescription market was around two to four times of that size. Lot of products are being developed to combat acne such as topical retinoids, benzoyl peroxide, salicylic acid etc. but due to high prevalence of antibiotic resistant strains of *Propionibacterium acnes*, topical antibiotics are no longer effective as monotherapy².

Mixture of Benzoyl Peroxide and Erythromycin can be beneficial as they exert a synergistic effect on skin³. Benzoyl Peroxide inhibits the formation of free fatty acids in the skin, primarily through inactivation of extracellular lipase (via oxidation)⁴. Erythromycin effectively reduces the concentration of *P. acne*⁵.

Gels containing a combination of erythromycin and benzoylperoxide are frequently used in the treatment of acne vulgaris⁶. Erythromycin is a macrolide antibiotic produced by *Saccharopolyspora erythraea* during fermentation⁷. The chemical structure of EA is shown in Fig. 1. Erythromycin is used in the treatment of acne because of its bacteriostatic activity against *Propionibacterium acnes*, which can be found in the sebum. Benzoylperoxide acts as a keratolytic⁸ and also has antibacterial activity against *Propionibacterium acnes* because of its oxidative power. The chemical structure of benzoylperoxide is shown in Fig. 2. Analytical methods for the quantitative determination of Benzoyl peroxide and Erythromycin in pharmaceutical formulations described in the literature is by liquid chromatography⁹ and microbiological method respectively. The aim of this work was to develop UV methods for both compounds. Solutions containing both erythromycin and benzoylperoxide are not stable because erythromycin is readily oxidized and derivatised by benzoylperoxide. Therefore sample preparation is more difficult than usual. Here benzoyl peroxide is detected by UV spectrophotometer since Erythromycin has weak chromophore which is not well detected at lower concentration so for analyzing Erythromycin, solution is allowed to react with concentrated H₂SO₄ due to which it gives yellow color because the sugar moiety of

Erythromycin react with H₂SO₄ and shows maximum absorbance at 480nm. At this wavelength Benzoyl Peroxide doesn't show any absorbance.

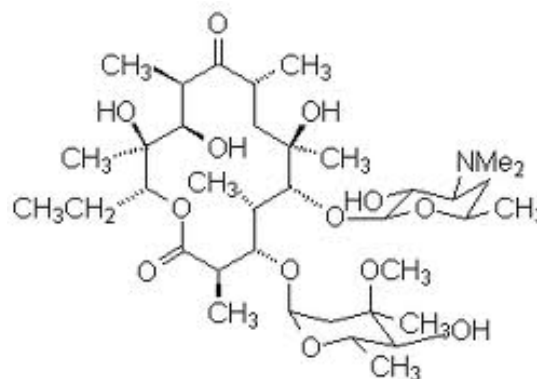


Fig. 1: Structure of Erythromycin

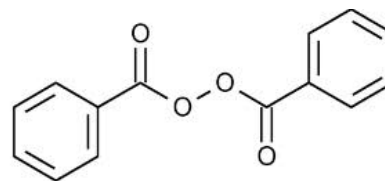


Fig. 2: Structure of Benzoyl Peroxide

MATERIAL & METHOD

Erythromycin and Benzoyl peroxide was kindly supplied as gift sample by AnuhPharma Ltd. Mumbai. Acetonitrile and water used were of Spectroscopy grade. H₂SO₄ was purchased from SR Chemicals. To prepare gel containing 3% erythromycin and 5% benzoyl peroxide carbopol 940, docusate sodium, sodium hydroxide, methyl salicylate and ethanol were purchased from SR chemicals.

Preparation of Gel

Carbopol was added to purified water with stirring. Stirring of mixture was done for 40 minutes. Then sodium hydroxide dissolved in water was added to mixture and stirred for 10 minutes. After addition of methyl salicylate, benzoyl peroxide was added. The mixture was stirred for 30 minutes till elegant and smooth gel was obtained. Erythromycin was dissolved in Ethanol and added to above mixture and stirred for 15 minutes. Gel prepared store in suitable container.

1. Preparation of calibration curve of benzoyl peroxide in acetonitrile:water(1:1)

Reagents

1. Freshly prepared acetonitrile: water(1:1) mixture.
2. Stock solution of benzoyl peroxide: 100 µg/ml solution of benzoyl peroxide was prepared in acetonitrile:water (1:1) mixture.

Method

Stock solution was further diluted to prepare different strength of standard solution. Aliquots of stock solution of benzoyl peroxide were transferred into a series of 10 ml volumetric flasks and volume was made up to the mark to produce the concentration ranging from 1-10 µg/ml. From the UV absorption spectra 236 nm was selected as detection wavelength.

Calibration curve

Calibration curve was plotted in the concentration range of 1-10 µg/ml by diluting the standard stock solution with acetonitrile: water (1:1) mixture. The absorbance was measured at 236 nm against the corresponding solvent blank. The linearity of the plot between absorbance and the concentration of the drug in the concentration range 1-10 µg/ml was calculated to obtain the calibration curve as well as the regression coefficient (r^2). The absorption of all the prepared solutions was measured at the absorbance maxima of 236 nm against the reagent blank. The readings were recorded.

2. Preparation of calibration curve of erythromycin in acetonitrile:water (1:1) mixture

Reagents

1. Freshly prepared acetonitrile:water(1:1) mixture.
2. Stock solution of ERYTHROMYCIN: 500 µg/ml
3. Conc. H₂SO₄

Method

The method employed use of acetonitrile:water (1:1) mixture as a solvent and use of concentrated H₂SO₄ to derivatives Erythromycin. Completion of reaction was ensured by keeping the samples at 50° C for 30 minutes. Sugar moiety of erythromycin reacts with conc. H₂SO₄ to give yellow color¹⁰. From the stock solution 50ppm, 100ppm, 200ppm, 300ppm, 400ppm and 500ppm solution was prepared and 1ml from each was added to volumetric flask containing 1ml of conc. H₂SO₄ and kept at 50° C for 30 minute. Then volume was made up to 10ml therefore the final concentrations were 5, 10, 20, 30, 40 and 50ppm. From the UV absorption spectra 480 nm was selected as detection wavelength.

3. Analytical method for estimation of benzoyl peroxide in their combined dosage form.

Reagents

1. Freshly prepared acetonitrile:water (1:1) mixture.
2. Stock solution containing benzoyl peroxide and erythromycin: 25mg Erythromycin and 50mg Benzoyl peroxide dissolved in 50 ml solvent.

Method

UV spectroscopic method was used which was similar to method described earlier in 1. Absorption wavelength of 236 nm was taken under consideration where erythromycin gave no interference or zero absorbance since erythromycin has weak chromophore. Benzoyl Peroxide showed good linearity in presence and absence of erythromycin at that wavelength. So that wavelength can be used for estimation of benzoyl peroxide in combined dosage form.

4. Analytical method for estimation of Erythromycin in their combined dosage form.

Reagents

1. Freshly prepared acetonitrile:water(1:1) mixture.

2. Stock solution containing benzoyl peroxide and erythromycin: 25mg Erythromycin and 50mg Benzoyl peroxide dissolved in 50 ml solvent.
3. Conc. H₂SO₄

Method

UV spectroscopic method was used which was similar to method described earlier in 2. Absorption wave length of 480 nm was taken under consideration where Benzoyl Peroxide gave no interference at this wavelength. Erythromycin showed good linearity in presence and absence of Benzoyl peroxide at that wavelength. So this wavelength can be used for estimation of Erythromycin in combined dosage form.

Validation of method by ICH guideline^{11, 12, 13}

The method was validated according to the guidelines set on the International Conference on Harmonization (ICH) for the validation of the analytical procedures. The parameters, which were used to validate the method, were linearity, range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity and robustness. The linearity response was assessed in the range of 5-25 and 1-10 µg/ml for Erythromycin and Benzoyl Peroxide respectively. The linearity was evaluated by calculation of the correlation coefficient obtained from linear regression analysis. The repeatability of the analytical method was assessed by assaying six sample solutions of Erythromycin and Benzoyl Peroxide during same day under the same experimental conditions. Intermediate precision was evaluated by analyzing the solutions on three different days. Absorbance was determined and compared. Precision was expressed as percentage relative standard deviation (%RSD).

Specificity

The specificity of the method was investigated by observing any interference encountered from any excipients of the gel. It was found that these excipients did not interfere with the proposed method.

Linearity and Range

The analytical concentration ranges over which the drugs obeyed Beer Lambert's law, were found to be 1-10 µg/ml for Benzoyl peroxide and 5-25 µg/ml for Erythromycin. The standard calibration curve is given in figure 3-4.

Precision

Precision was studied to find out intra and inter-day variation in the test method of Benzoyl peroxide and Erythromycin. Calibration curves prepared in solvent were run in triplicate in same day for three days.

Limit of detection and quantification

Determination of the detection and quantification limits was performed based on the standard deviations of the blank.

Recovery Study

To study the accuracy of the proposed method, recovery study was carried out by standard addition method at three different levels. A known amount of drug was added to preanalyzed gel and percentage recoveries were calculated.

Also the accuracy and Precision of the developed method was evaluated on gel through recovery test by standard addition method. In this two stock solution were prepared one for analysis of Benzoyl Peroxide and one for Erythromycin of 100 µg/ml and 500 µg/ml concentration respectively from gel containing 3% Erythromycin and 5% Benzoyl Peroxide using solvent acetonitrile and water (1:1).

RESULT AND DISCUSSION

From the diffusion point of view, attempt was made to choose solvent system in which both drugs were soluble. The calibration curve of Erythromycin and Benzoyl Peroxide was plotted at 480nm and 236 nm respectively (Table 1-2, Fig. 3-4).

Table 1: Calibration curve data for benzoyl peroxide

Concentration ($\mu\text{g/ml}$)	Absorbance						Average	%RSD
	I	II	III	IV	V	VI		
1	0.1087	0.1086	0.1088	0.1088	0.1086	0.1087	0.1088	0.082696
2	0.2332	0.2331	0.2333	0.2333	0.2331	0.2333	0.233217	0.038485
4	0.4342	0.4344	0.4345	0.4343	0.4344	0.4342	0.434333	0.025454
6	0.6358	0.6357	0.6357	0.6358	0.6359	0.6356	0.63575	0.01506
8	0.8766	0.8765	0.8764	0.8766	0.8765	0.8766	0.876533	0.008503
10	1.0819	1.0818	1.0819	1.0818	1.082	1.0819	1.081883	0.006352

Table 2: Calibration curve data for erythromycin.

Concentration ($\mu\text{g/ml}$)	Absorbance						Average	%RSD
	I	II	III	IV	V	VI		
5	0.0442	0.0441	0.0441	0.0443	0.0442	0.0442	0.044183	0.170375
10	0.0837	0.0837	0.0835	0.0836	0.0837	0.0835	0.083617	0.117583
15	0.1272	0.1275	0.1273	0.1274	0.1276	0.1274	0.1274	0.111006
20	0.164	0.1643	0.1644	0.1645	0.1644	0.1643	0.164317	0.104822
25	0.2014	0.2012	0.2015	0.2015	0.2013	0.2011	0.201333	0.081109

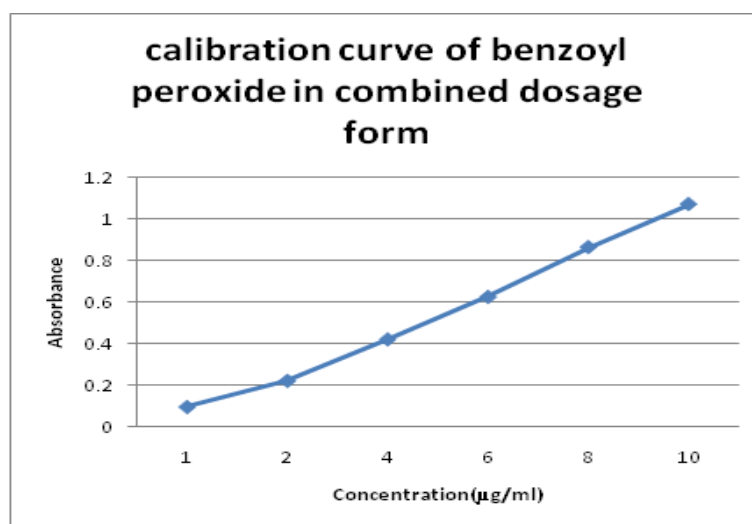


Fig. 3: Calibration curve data for benzoyl peroxide for the estimation of benzoyl peroxide in combined dosage (n=6)

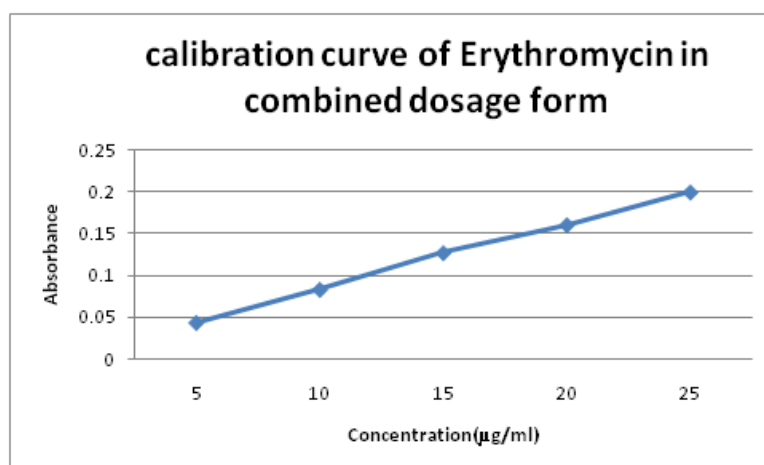


Fig. 4: Calibration curve data for erythromycin for the estimation of erythromycin in combined dosage (n=6)

The proposed methods for simultaneous estimation of erythromycin and benzoyl peroxide in combined dosage form were found to be accurate, simple and rapid which can be well understood from validation data as given in Table 3-9. The % R.S.D. as indicated in Table 9 was found to be less than 2, which indicates the validity of methods. Linearity was observed by linear regression equation method for Erythromycin and benzoyl

peroxide in different concentration range. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. Precision was calculated as repeatability, inter and intraday variations and % RSD was less than 1 for both drugs. The LOD value was found to be 0.0881 and 1.4358 while LOQ value was found to be 0.2672 and 3.7341 for Benzoyl Peroxide and Erythromycin respectively.

Table 3: Summary of optical characteristics benzoyl peroxide and erythromycin

Characteristic	Values for Benzoyl Peroxide	Values for Erythromycin	Values for Benzoyl Peroxide For combination of both Drugs	Values for Erythromycin For combination of both Drugs
λmax	238nm	480nm	238nm	480nm
Solvent	Acetonitrile	Acetonitrile	Acetonitrile	Acetonitrile
Range	water(1:1) 1-10µg/ml	water(1:1) 5-25µg/ml	water(1:1) 1-10µg/ml	water(1:1) 5-25µg/ml
Regression equation	y = 0.199x - 0.137	y = 0.039x + 0.005	y = 0.199x - 0.147	y = 0.039x + 0.005
Regression Coefficient (r ²)	0.997	0.9991	0.992	0.9989

Table 4: Precision study for benzoyl peroxide

Time	Inter day absorbance for 2µg/ml concentration						Mean	%R.S.D.
	I	II	III	IV	V	VI		
Day1	0.2334	0.2335	0.2336	0.2336	0.2337	0.23335	0.23353	0.05705133
Day2	0.2345	0.2342	0.2346	0.2344	0.2345	0.2348	0.2345	0.08528785
Day3	0.2339	0.234	0.2341	0.2346	0.2345	0.2346	0.23428	0.13609679
Time	Intraday absorbance for 4µg/ml concentration						Mean	% R.S.D.
	I	II	III	IV	V	VI		
Morning	0.4341	0.4345	0.4346	0.4346	0.4348	0.4342	0.43446667	0.06118583
Afternoon	0.4342	0.4344	0.4345	0.4343	0.4344	0.4342	0.43433333	0.0278832
Evening	0.4446	0.4448	0.4441	0.4446	0.4439	0.4441	0.44435	0.08145363

Table 5: Precision study for erythromycin

Time	Inter day absorbance for 5µg/ml concentration						Mean	%RSD
	I	II	III	IV	V	VI		
Day1	0.0452	0.0453	0.0458	0.0455	0.0454	0.0456	0.04546667	0.47512762
Day2	0.0449	0.0448	0.0453	0.0451	0.0455	0.0456	0.0452	0.71347414
Day3	0.0455	0.0457	0.0458	0.0457	0.0458	0.0455	0.04566667	0.29918104
Time	Intraday absorbance for 15µg/ml concentration						Mean	%RSD
	I	II	III	IV	V	VI		
Morning	0.1248	0.1244	0.1246	0.1249	0.1247	0.1244	0.12463333	0.16573344
Afternoon	0.1255	0.1252	0.1255	0.1253	0.1255	0.1253	0.12538333	0.10600772
Evening	0.1276	0.1279	0.1276	0.1274	0.1274	0.1273	0.12753333	0.16938685

Table 6: Recovery study for benzoyl peroxide

Sr. No.	Fix conc. Taken (µg/ml)	Conc. Added (µg/ml)	% added (µg/ml)	Total conc. (µg/ml)	Concobt (µg/ml)	Conc recovered (µg/ml)	% Recovered
1	1	0.8	80	1.8	1.79	0.79	98.75
2	1	1	100	2	1.98	0.98	98
3	1	1.2	120	2.2	2.21	1.21	100.83

Table 7: Recovery study for erythromycin

Sr. No.	Fix conc. Taken (µg/ml)	Conc. Added (µg/ml)	% added	Total conc. (µg/ml)	Conc. obtain (µg/ml)	Conc. recovered (µg/ml)	% Recovered
1	10	8	80	18	17.91	7.91	98.87
2	10	10	100	20	20.04	10.04	100.4
3	10	12	120	22	22.14	12.14	101.16

Table 8: Recovery study for erythromycin and benzoyl peroxide gel formulation

Drug	Theoretical Concentration (µg/ml)	Mean of Calculated Concentration (µg/ml) n=3	% Accuracy
Erythromycin	15	15.26	101.01
Benzoyl peroxide	4	3.91	97.75

Table 9: Intraday and interday precision for erythromycin and benzoyl peroxide gel formulation

Drug	Erythromycin		Benzoyl Peroxide	
	Intraday precision	Interday precision	Intraday precision	Interday precision
%RSD	0.94	0.96	0.41	0.40

ACKNOWLEDGEMENT

The authors thank AnuhPharma Ltd., Mumbai, for providing gift sample for this work. Also thanks to HSNC board for providing required facilities to carry out this research work. I m thankful to Mr. SajitKasbeand Mr. KundanBadhe for helping me in my research work.

REFERENCES

1. Robert W. Klein, Mary E. Foxx Inventors, Dermik Laboratories, Inc. , assignee, Erythromycin/ benzoyl peroxide composition for the treatment of acne, United State Patent. US patent 4,497,794, 1985 Feb5.
2. Naithani V, Kumar S, Srivastava S, Pathak N, International Journal of Drug Development & Research 2010; 2(1):134-140.
3. Lisa W., Ronald B., Topical Approaches in Combination Therapy for acne, Skin, Therapy Letter 2011, 7(5), 1-12
4. Vishnupad M, Vishnupad N, Inventors, Imaginative research Associates, Assignee; Antibiotic/Benzoyl Peroxide Dispenser, United State Patent, US Patent 7,060,732 B2, 2006 June13.
5. Jain A, Sangal L, Basal E, Kaushal GP and Agarwal Sk, Anti-inflammatory effects of Erythromycin and Tetracycline on *Propionibacterium acnes* induced production of chemotactic factors and reactive oxygen species by human neutrophils, Dermatology Online Journal 8(2): 2
6. David CL, Management of acne, The Journal of family Practice 2003; 52:1
7. Dehouck P, Heyden Y, Massart D, Marini R, Chiap P, et al., Interlaboratory study of a liquid chromatography method for erythromycin: determination of uncertainty, Journal of Chromatography A 2003; 1010: 63-74
8. Dehoucka P, Looya E, Haghedoorena E, Deckers K, Vander, Adamsa E, et. al., Analysis of erythromycin and benzoylperoxide in topical gels by liquid chromatography, Journal of Chromatography B, 2003; 794(2): 293-302
9. Waller JM, Dreher F, Behnam S, Ford C, Lee C, Tiet T et al. 'Keratolytic' properties of benzoyl peroxide and retinoic acid resemble salicylic acid in man, Skin Pharmacol Physiol. 2006; 19(5): 283-9
10. Danielson N, Holeman J, Bristol D, Kirzner D, Simple methods for the qualitative identification and quantitative determination of macrolide antibiotics, Journal of Pharmaceutical & Biomedical Analysis, 1993; 11(2) :121-130.
11. ICH - Q2 (R1) (2005) Guideline on Validation of Analytical Procedure. Text and Methodology.
12. Raju MT, Gurralla SG, Rao J.V., Rao KRS, Development and validation of HPLC-UV method for the estimation of Eprosartan in human plasma, Int J Pharm Pharm Sci, 2011; 3(2): 191-194
13. Delvadiya K, Kimbahune R, Kabra P., Patel P., Spectrophotometric simultaneous analysis of paracetamol, propyphenazone and caffeine in tablet dosage forms, Int J Pharm Pharm Sci, 2011; 3(3): 170-174.