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A VALIDATED UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TRETINOIN AND BENZOYL PEROXIDE IN BULK AND SEMI SOLID DOSAGE FORM

Ankush Gupta, Monica Gulati and Narendra Kumar Pandey*
*Department of Pharmaceutical sciences, Lovely Professional University,
Chehru, Phagwara, Punjab-144402, India
E-mail: herenarendra4u@gmail.com

ABSTRACT

A simple, sensitive, spectrophotometric method in UV region has been developed for the simultaneous estimation of benzoyl peroxide and tretinoin in bulk and semi solid dosage form (cream). Standard solution of benzoyl peroxide shows maximum absorbance at 234.8 nm and tretinoin shows maximum absorbance at 348.6 nm. Beer's Lamberts law is obeyed in concentration range 1-9 µg/ml for tretinoin with regression, slope and intercept of 0.991, 0.0918 and 0 respectively while for benzoyl peroxide, Beer's Lambert law is obeyed in concentration range 0.5-5 µg/ml with regression, slope and intercept having 0.9984, 0.2069 and 0.0253 respectively. Method was validated for linearity, range, accuracy, precision, recovery studies and interference study of mixture. All these parameters showed the adaptability of the method for the quality control analysis of the drug in bulk and in combination formulations.

Keywords: Tretinoin, benzoyl peroxide, semisolid dosage form cream, absorbance interference study.

INTRODUCTION

Tretinoin (3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)-nona-2,4,6,8-tetraenoic acid) is a vitamin A derivative used topically for the treatment of acne vulgaris[1]. It is yellow coloured amorphous powder, relatively insoluble in water and freely soluble in methanol[2] as benzoyl peroxide is a potent antibacterial agent which is the first line drug in treatment of acne vulgaris[3]. The chemical structure[2] of tretinoin and benzoyl peroxide is shown in fig1 and fig2.

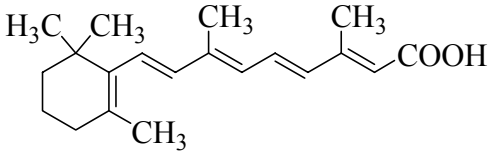

Fig.-1: Chemical structure of tretinoin

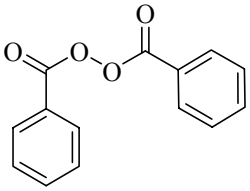

Fig.-2: Chemical structure of benzoyl peroxide (BPO)

The combination of both drugs would be beneficial for the treatment of mild to moderate stage of acne vulgaris[4]. However for the combination therapy of two drugs if they are administered in the form of a

single formulation, a simultaneous estimation of the two would be required. Literature survey revealed that there was no validated UV method for the estimation of tretinoin and benzoyl peroxide simultaneously by UV spectrophotometric method in bulk and semisolid dosage form. The present study is aimed to develop selective, precise accurate and reliable UV method for determination of BPO and tretinoin in mixture of bulk and semi solid dosage form.

EXPERIMENTAL

Instrument and reagents:

A Systronic UV-Visible Spectrophotometer 2201, with 1 cm matched quartz cell was used for the absorbance measurement. All the chemicals used were of analytical grade. Pure tretinoin and benzoyl peroxide obtained from Shalaks Pharmaceutical, New Delhi and H.K group, Mumbai respectively.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving tretinoin in methanol to make final concentration of 10µg/ml. Different aliquots were taken from stock solution and diluted with methanol to prepare series of concentration from 1-9 µg/ml. The λ_{max} was found to be 348.6 nm in methanol (fig6). Absorbance was measured at 348.6 nm against methanol as a blank. The calibration curve was prepared by plotting absorbance versus concentration of tretinoin (fig3). The same procedure was used for benzoyl peroxide vice versa but dilution from stock solution was prepared from 0.5-5 µg/ml using ethanol as solvent. The λ_{max} was found to be 234.8 nm (fig7). Absorbance was measured at 234.8 nm using ethanol as blank and calibration curve was prepared (fig4).

Multicomponent scan analysis for interference study in mixture of bulk

Prepared a stock solution of benzoyl peroxide (10µg/ml) in ethanol and also prepared the stock solution of tretinoin (10µg/ml) in methanol. Took 5ml of each of stock solution and mixed it thoroughly, then scanned the resultant mixture of tretinoin and benzoyl peroxide from 200-400 nm using UV spectrophotometer (fig5).

Application of the proposed procedure for the determination of API's in cream

The proposed procedure was adopted for the determination of tretinoin and benzoyl peroxide in cream. 200 mg of cream (2.5% benzoyl peroxide and 0.025% tretinoin) dissolved in 100 ml of 1:1 mixture of ethanol and methanol. Then the solution was filtered through Whatmann filter paper. Then the concentration of benzoyl peroxide and tretinoin was calculated in the resultant solution. The readings were taken in triplicate.

Accuracy and Precision

Accuracy and precision were investigated by analyzing three concentration of tretinoin and benzoyl peroxide mixture in three independent replicates on the same day (Intra-day accuracy and precision) and on three consecutive days (Inter-day accuracy and precision). Intra-day and Inter-day relative standard deviation was calculated[5].

Recovery Studies

The standard addition technique was carried out by adding excipients, with the addition of 50%, 100% and 140% of drugs respectively in sample solution. The percentage recoveries of three concentrations were calculated.

Robustness

Robustness was tested by making deliberate small changes in concentration of solvent used in estimation of API's and observed the changes in various parameters like absorbance maximum, Beer's law limit, slope, intercept and correlation coefficient[5].

RESULTS AND DISCUSSION

Statistical evaluation of analysis and recovery studies were carried out. The data obtained from the proposed method showed accuracy of method. The values of standard deviation were satisfactorily low. The percent recovery of 99% was indicative of accuracy of method.

Validation of Method

The method was validated with respect to linearity and range, accuracy and precision, interference study, recovery studies and robustness.

Linearity and range

The prepared aliquots for tretinoin (1-9µg/ml) were scanned for absorbance at λ_{max} 348.6 nm. The absorbance range was found to be 0.08- 0.813. These aliquots obeyed Beer-Lambert's law with regression of 0.9991 and for benzoyl peroxide the aliquots prepared (0.5-5 µg/ml) were scanned for absorbance at λ_{max} 234.8 nm. Absorbance range was found to be 0.085-1.017 with regression of 0.9984. The optical characterstic are shown in Table 1.

Accuracy and Precision

The data evaluated has been summarized in Table 2 and Table 3. Intra-day and Inter-day relative standard deviation values and the low RSD value obtained from the analysis of pharmaceutical formulations indicated good precision of the method.

Absorbance interference study

The characterstic peak for tretinoin was obtained at 348.6 nm and for benzoyl peroxide it was obtained at 234.8. For check out the interference of absorbance of one component with other component the absorbance of tretinoin was seen at 234.8 nm it was -0.059 and for benzoyl peroxide at 348.6 nm it was -0.026. The result showed that there was no interference in the absorbance of each component in the mixture.

Recovery Studies

The percentage recoveries of three concentrations were found to be close to 100%, indicative of high accuracy. The high percent recoveries indicate no interference from ingredients and excipients that might be present in formulation Table 4 and Table 5.

Robustness

Repeatability is based on the results of the method operating over short time interval under same conditions. The low RSD value of intra-day precision, recovery and pharmaceutical preparation showed high repeatability.

ACKNOWLEDGEMENTS

The authors are thankful to M/s Shalaks Pharmaceutical, New Delhi and H.K group, Mumbai for providing gift sample of tretinoin and benzoyl peroxide respectively.

Table-1: Optical characterstics for tretinoin and benzoyl peroxide (BPO)

Parameter	Tretinoin	Benzoyl peroxide
Absorbance maximum (λ_{max})	348.6 nm	234.8 nm
Beer's law limit (µg/ml)	1-9	0.5-5
Correlation coefficient (r^2)	0.9991	0.9984
Slope	0.0918	0.2069
Intercept	0	0.0253

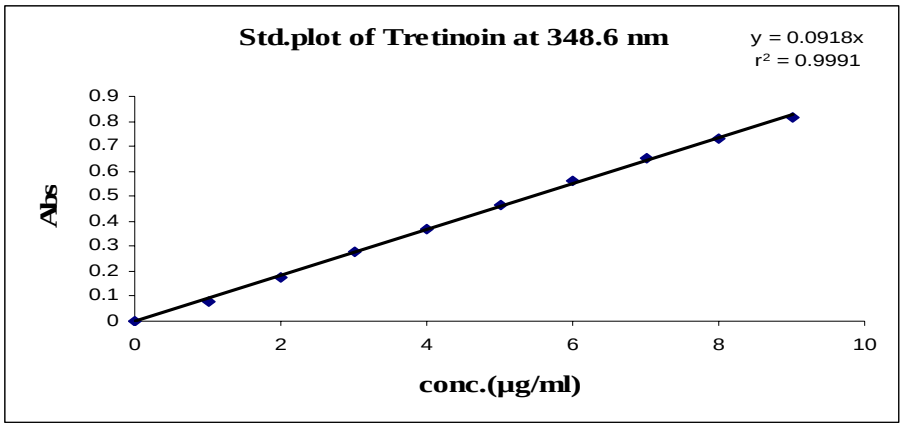

Fig.-3: Standard Calibration Curve of Tretinoin at 348.6 nm

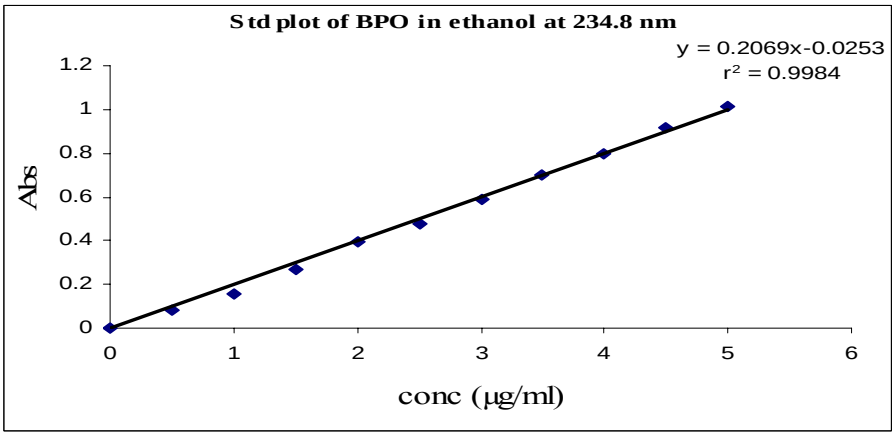

Fig.-4: Standard Calibration Curve of BPO at 234.8 nm

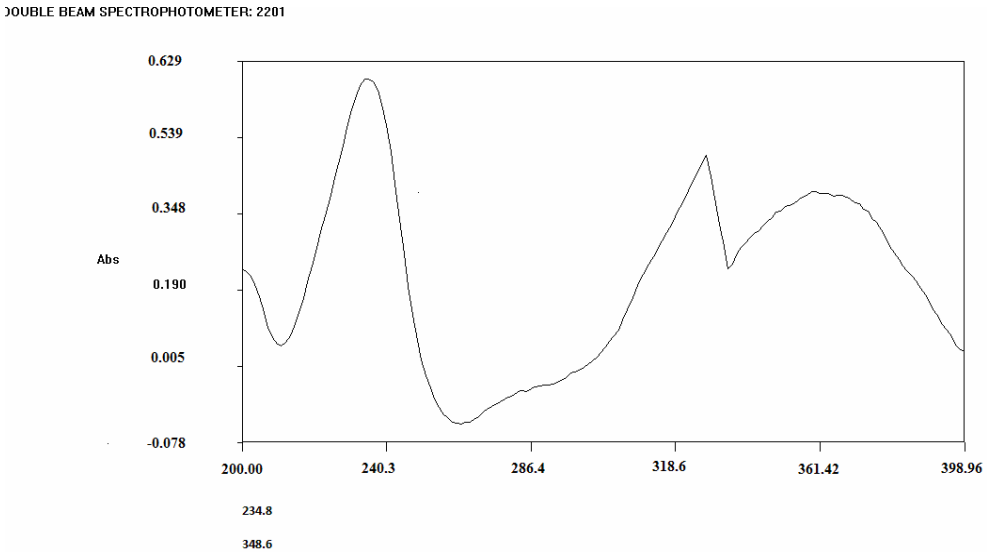

Fig-5: UV Absorption Spectra of Benzoyl Peroxide and Tretinoin mixture of Bulk

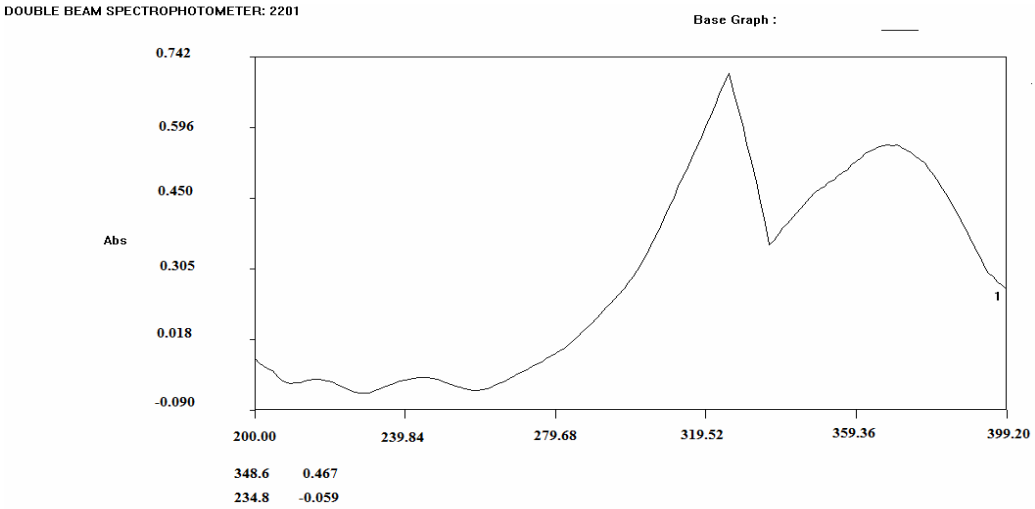

Fig.-6: UV Absorption Spectra of Tretinoin

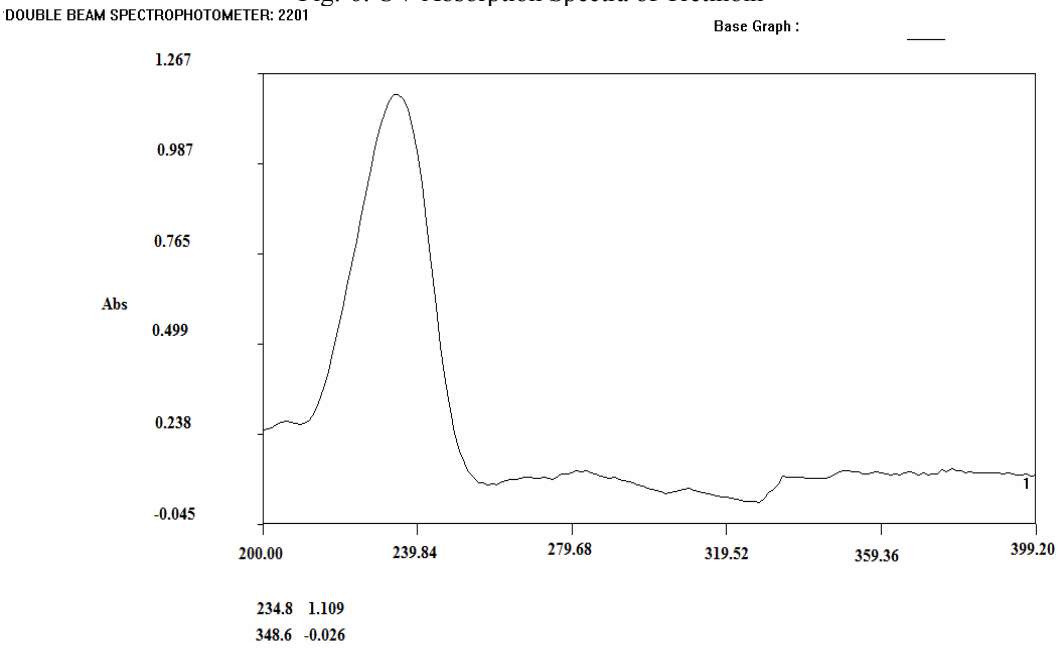

Fig.-7: UV Absorption Spectra of Benzoyl Peroxide

Table-2: Results of accuracy and precision for Tretinoin

Amount of drug (µg)	Avg. amount found in Intra days studies (µg)	Avg. amount found in Inter days studies (µg)	Precision (Intra day)*	Precision (Inter day)*
25	24.8	24.85	0.213	0.212
50	49.67	49.70	0.050	0.334
75	74.56	74.73	0.306	0.096

** Percentage RSD of three samples*

Table-3: Results of accuracy and precision for benzoyl peroxide

Amount of drug (mg)	Avg. amount found in Intra days studies (mg)	Avg. amount found in Inter days studies (mg)	Precision (Intra day)*	Precision (Inter day)*
25	24.74	24.76	0.366	0.352
50	49.79	49.80	0.345	0.398
75	74.77	74.74	0.144	0.136

** Percentage RSD of three samples*

Table-4: Results of recovery studies for tretinoin

% addition of drug	Amount of drug (µg)	Avg. amount found in Intra days studies (µg)	Avg. amount found in Inter days studies (µg)	Precision (Intra day)*	Precision (Inter day)*
0	25	24.90	24.98	0.465	0.424
50	37.5	37.38	37.43	0.126	0.259
100	50	49.77	49.72	0.297	0.212
140	60	59.98	59.72	0.336	0.265

** Percentage RSD of three samples*

Table-5: Results of recovery studies for benzoyl peroxide

% addition of drug	Amount of drug (mg)	Avg. amount found in Intra days studies (mg)	Avg. amount found in Inter days studies (mg)	Precision (Intra day)*	Precision (Inter day)*
0	25	24.77	24.82	0.387	0.369
50	37.5	37.45	37.40	0.202	0.173
100	50	49.86	49.68	0.128	0.164
140	60	59.85	59.87	0.076	0.142

** Percentage RSD of three samples*

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