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Estimation of metronidazole benzoate in bulk and formulation by first order derivative area under curve UV-spectrophotometric methods

[*1] Audumbar Mali, [2] Ankush Hake, [2] Manoj Patil, [1] Ritesh Bathe, [1] Manojkumar Patil, [3] Ashpak Tamboli

[*1] Department of Pharmaceutics, Sahyadri College of Pharmacy, Methwade, Sangola, Solapur, aharashtra, India.

[2] Department of Pharmaceutical Chemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra, India.

[3] Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, Solapur, Maharashtra, India.

Abstract
Simple, fast and reliable spectrophotometric methods were developed for determination of Metronidazole Benzoate in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in 0.1 N HCl. The quantitative determination of the drug was carried out using the first order Derivative Area under Curve method values measured at 236-251nm. Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Metronidazole Benzoate using 5-25µg/ml (r^2=0.9994) for first order derivative Area under Curve spectrophotometric method. The proposed methods have been extensively validated as per ICH guidelines. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations. The developed methods were successfully applied to estimate the amount of Metronidazole Benzoatein pharmaceutical formulations.

Keywords: Metronidazole Benzoate, First order derivative, Area under Curve (AUC), Precision, Accuracy.

1. Introduction

Chemically Metronidazole benzoate is 2-(2-methyl-5-nitro-1H-imidazole-1-yl) ethyl benzoate, belonging to the category of antiprotozoal drug. The uses of metronidazole for antiprotozoal therapy have been reviewed extensively. It is clinically effective in trichomoniasis, amebiasis, and giardiasis, as well as in a variety of infections caused by obligate anaerobic bacteria, including Bacteroides, Clostridium, Fusobacterium, Peptococcus, Peptostreptococcus, Eubacterium, andmicroaerophilic bacteria such as Helicobacter and Campylobacter spp. Metronidazole antimicrobial properties are thought to derive from the formation of toxic-free radicals by intracellular reduction. [1, 2] In our Literature survey reveals that for Metronidazole Benzoate Spectrophotometric [3, 5] methods and HPLC [6, 11] methods have been reported for its determination in commercial formulation. To our notice, no UV-spectrophotometric method using First Order Derivative Area under Curve has been reported for the determination of Metronidazole Benzoatein bulk and tablets. Hence an attempt has been made to develop new First Order Derivative Area under Curve spectrophotometric method for estimation of Metronidazole Benzoate in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

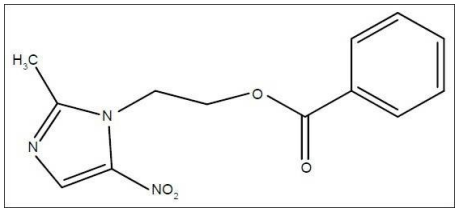

Fig 1: Structure of Metronidazole Benzoate

2. Materials and Methods: [12, 16]

2.1. Derivative Spectrophotometric Methods:-

The First derivative spectrophotometry was used in the wavelength ranges from 236 and 251 nm.

[dA/dλ= f(λ): first order

The First derivative spectrum of an absorption band is characterized by a maximum, a minimum, and a cross-over point at the λ max of the absorption band.

2.2. Area under curve (Area calculation)

In this study area was integrated between wavelength ranges from 236-251 nm.

Area calculation: $(\alpha+\beta) = \int_{\lambda 2}^{\lambda 1} A d\lambda$

Where, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ1 and λ2 are wavelength range start and end point of curve region.

2.3. Apparatus and instrumentation

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glassware (Borosil®) was used for the validation study.

2.4. Materials

Reference standard of Metronidazole Benzoate API was supplied as gift sample by Cipla Pharmaceutical Limited, Pune. Tablets sample with label claim 500 mg per tablet were purchased from local market Pune.

3. Method development [17, 19]

3.1. Preparation of Standard and Sample Solutions:-

Stock solution of 10µg/ml of Metronidazole Benzoatewas prepared in 0.1 N HCl, for First Order Derivative Area under Curve spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with 0.1 N HCl in a concentration range of 5, 10, 15, 20 and 25µg/ml with 0.1 N HCl for First Order Derivative Area under Curve spectrophotometric methods. 0.1 N HCl was used as a blank solution.

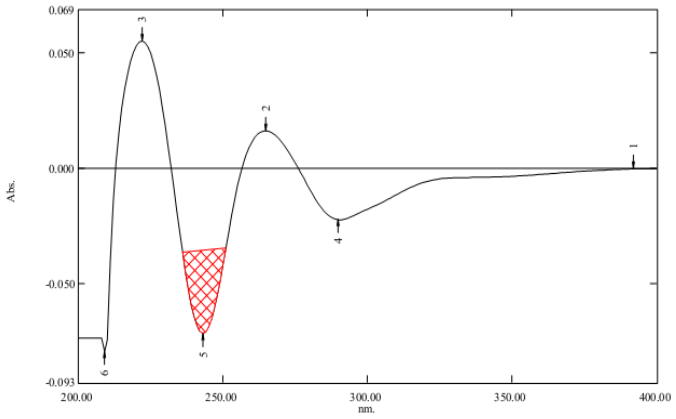

Fig 2: First order derivative Area under Curve spectrum of Metronidazole Benzoate in 0.1 N HCl (25µg/ml).

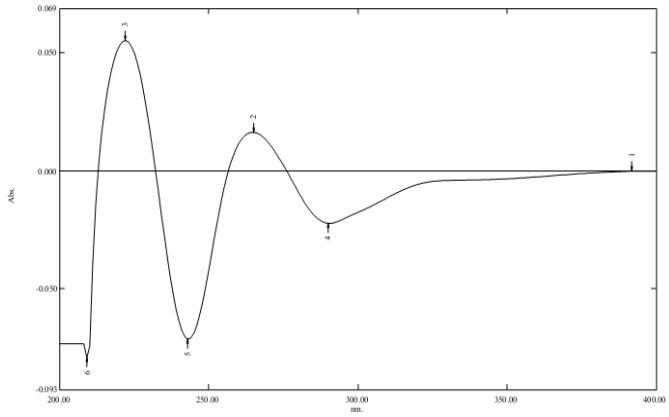

Fig 3: First order derivative spectrum of Metronidazole Benzoate in 0.1 N HCl (25µg/ml).

3.2. Calibration curve for Metronidazole Benzoate

The dilutions were made from Standard Stock solution to get concentration of 5, 10, 15, 20, and 25µg/ml respectively. These solutions were scanned from 400 to 200 nm and First Order Derivative Area under Curve values was integrated in the range of 236-251 nm. The calibration curve was plotted between areas under curve values against concentration.

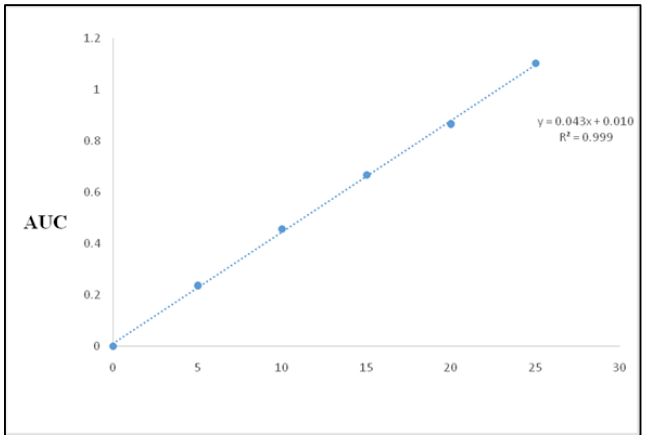

Fig. 4: Linearity of Metronidazole Benzoate.

3.3. Assay of tablet formulation

Twenty tablets each containing 500 mg of Metronidazole Benzoatewere weighed crushed to powder and average weight was calculated. Powder equivalent to 10 mg of Metronidazole Benzoatewas transferred in 100 ml of volumetric flask. A 50 ml of 0.1 N HCl was added and sonicated for 15 minutes. Then solution was further diluted up to the mark with 0.1 N HCl. The solution was filtered using Whatmann filter paper no. 41, first 5 ml of filtrate was discarded. This solution was further diluted to obtain 10µg/mL solution with water, subjected for UV analysis using 0.1 N HCl as blank. This procedure was repeated three times.

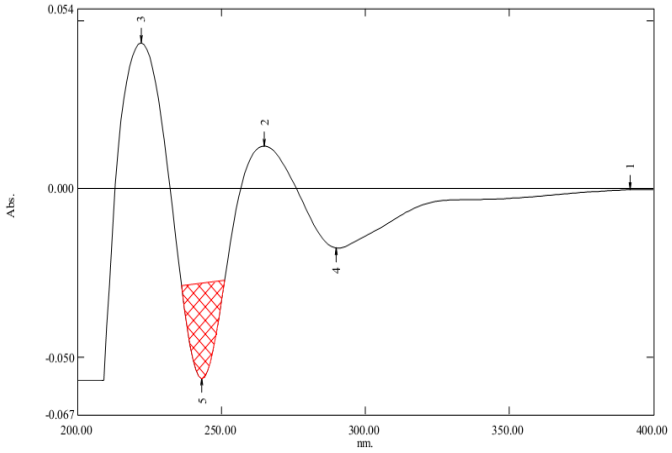

Fig 5: First order derivative Area under Curve spectrum of Metronidazole Benzoate of dosage form in 0.1 N HCl (25µg/ml).

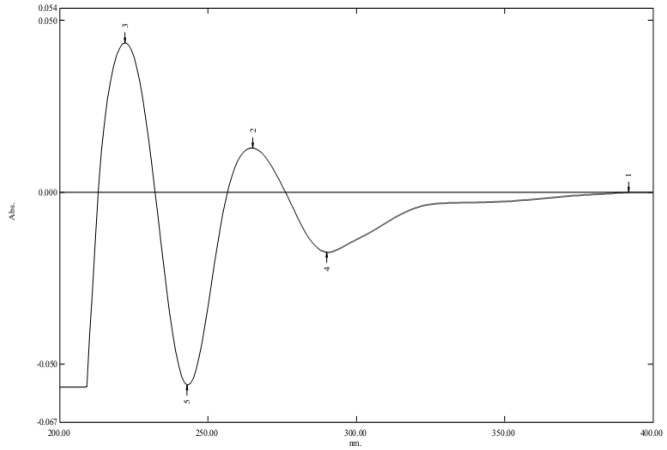

Fig 6: First order derivative spectrum of Metronidazole Benzoate of dosage form in 0.1 N HCl (25µg/ml).

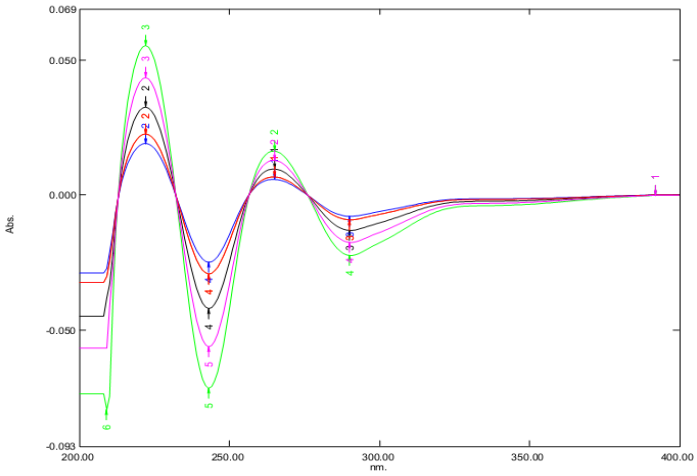

Fig 7: First order derivative overlay of Metronidazole Benzoateat different Concentration.

Table 1: Assay of tablet dosage form

Sr. No.	Sample Solution Concentration (µg/ml)	Amount found (%)	Mean % found*	%RSD*
1	25	99.12		
2	25	101.56	100.33	0.6518
3	25	100.32		

*n=3, % RSD = % Relative Standard Deviation.

4. Method Validation: [20, 22]

The above method was validated for various parameters such as Accuracy, Linearity, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH guideline.

4.1. Accuracy

The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 25µg/ml Sample solution. First Order Derivative Area under curve (AUC) was measured in wavelength range 236-251 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

Table 2: Accuracy results for Metronidazole Benzoate

Accuracy level	Sample conc (µg/ml)	Std. conc (µg/ml)	Total amount. Added (µg/ml)	% Recovery	Mean % Recovery	% RSD
80	25	12	22	102.65		
100	25	15	25	98.24	100.65	0.6891
120	25	18	28	101.08		

4.2. Precision

The precision of an analytical procedure expresses the closeness of an agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions intraday precision was studied by integrating area of standard solution of 25µg/ml concentration at six independent series in the same day. Interday precision studies were performed by integrating area of standard solution of 25µg/ml concentration on three consequent days. The % RSD was calculated.

Table 3: Precision Study

Parameter	Intra day	Inter-day
Sample sol conc. µg/ml	25	25
AUC (mean)	0.7452	0.7762
% RSD	0.9547	0.9851

4.3. Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

$LOD = 3.3\ \sigma / S$

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula

$LOQ = 10\ \sigma / S$

Where, σ is standard deviation of the response and

S is the slope of the calibration curve.

LOD& LOQ of Metronidazole Benzoatewas found to be 0.6428µg/ml &1.9281 µg/ml respectively.

Table 4: Summary of validation parameters

Parameter	Result
λ range	236-251
Regression Equation (Y=mx+c)	Y=0.0435x + 0.0109
Linearity range	5-25µg/ml
Slope	0.0435
Intercept	0.0109
Correlation coefficient (R^2)	0.9994
Limit of Detection (LOD) µg/ml	0.6428
Limit of Quantitation (LOQ) µg/ml	1.9281
Accuracy (Mean % Recovery)	100.65
Precision (% RSD)	0.6891

5. Results and Discussion

The UV visible spectroscopic method for the Metronidazole Benzoate by First order derivative Area under Curve was found to be simple, accurate, economical and reproducible. The drug concentrations were found to be linear in the range of 05-25 µg/ml and the correlation coefficient value of 0.9994 indicates that developed method was linear. For Precision the percent relative standard deviation (% RSD) was found to be 0.6891 while, intra-day and inter-day precision results in terms of percent relative standard deviation values were found to be 0.9547 and 0.9851 respectively thus the method is observed as precise. The accuracy of the method was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value is ≤ 2 indicates the accuracy of the method. The Limit of Detection and Limit of Quantitation values were found to be 0.6428 µg/ml & 1.9281 µg/ml respectively. The result of the analysis for pharmaceutical formulation by the developed method was consistent with the label claim, highly

reproducible and reliable. The method can be used for routine quality control analysis of Metronidazole Benzoate in bulk and pharmaceutical formulations.

6. Conclusion

The UV spectroscopic AUC method for the analysis ofMetronidazole Benzoate by First order derivative Area under Curve was found to be simple, precise, and accurate; can be used for assay of bulk drug and pharmaceutical dosage formulations.

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