

Estimation of ampicillin trihydrate in bulk and formulation by first order derivative area under curve UV-Spectrophotometric methods

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Abstract

Simple, fast and reliable spectrophotometric methods were developed for determination of Ampicillin Trihydrate in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in Methanol. The quantitative determination of the drug was carried out using the second order Derivative Area under Curve method values measured at 224-231nm. Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Ampicillin Trihydrate using 5-25µg/ml ($r^2=0.997$) 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 Ampicillin Trihydrate in pharmaceutical formulations.

Keywords: Ampicillin Trihydrate, First order Derivative, Area under Curve (AUC).

1. Introduction

Chemically Ampicillin is a glucocorticoid and its IUPAC name is (2S, 5R, 6R)-6-[(2R)-2-amino-2-phenylacetamido]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2 carboxylic acid. Ampicillin trihydrate¹ is an antibiotic active against mainly gram positive bacteria and some gram negative bacteria.[1,2] It is used for the treatment of infections due to streptococci and also used in urinary tract infections and respiratory tract infections.[3,4] It is soluble in dilute solutions of acids and alkali hydroxides.[5] In our Literature survey reveals that for Ampicillin Trihydrate Spectrophotometric[6] methods and HPLC[7] 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 Ampicillin Trihydrate in bulk and tablets. Hence an attempt has been made to develop new First Order Derivative Area under Curve spectrophotometric method for estimation of Ampicillin Trihydrate in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

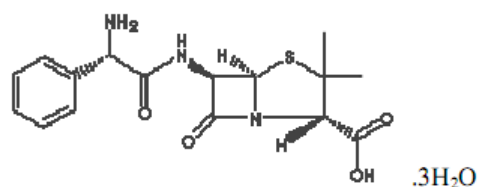


Fig. 1: Structure of Ampicillin Trihydrate

2. Materials and Methods

2.1 Derivative Spectrophotometric Methods

The first derivative spectrophotometry was used in the wavelength ranges from 241 and 251 nm. $[dA/d\lambda = f(\lambda)]$: 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.[8]

2.2 Area under curve (Area calculation):-

In this study area was integrated between wavelength ranges from 241 & 251 nm.

$$\text{Area calculation: } (\alpha + \beta) = \int_{\lambda_2}^{\lambda_1} A d\lambda$$

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, λ_1 and λ_2 are wavelength range start and end point of curve region.[9]

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 Ampicillin Trihydrate API was supplied as gift sample by Lupin Laboratory

Park, Aurangabad. Methanol was obtained from Research-Lab Fine Chem Industries, Islampur, Mumbai, and Maharashtra. Capsule sample with label claim 500 mg per Capsule were purchased from local market Mangalwedha, Solapur, Maharashtra, India.

2.5 Method development

2.5.1 Preparation of Standard and Sample Solutions

Stock solution of 10 μ g/ml of Ampicillin Trihydrate was prepared in Methanol, for First Order Derivative Area under Curve spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with Methanol in a concentration range of 5, 10, 15, 20 and 25 μ g/ml with Methanol for First Order Derivative Area under Curve spectrophotometric methods. Methanol was used as a blank solution.

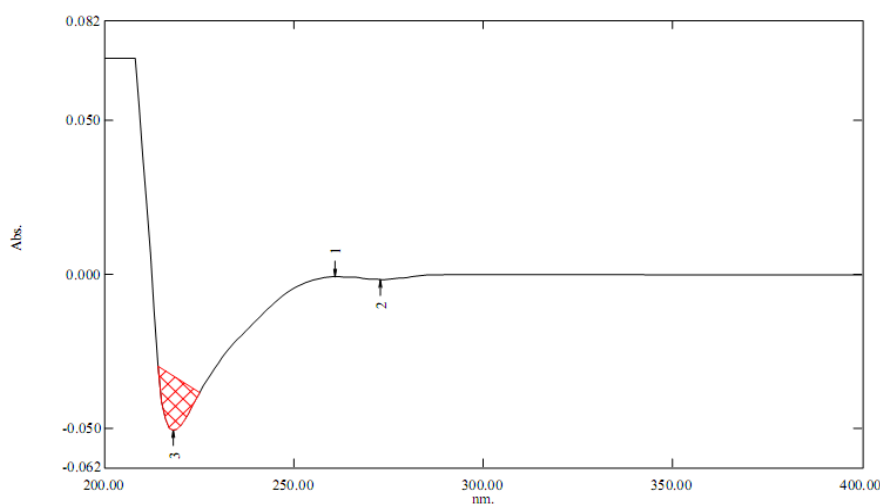


Fig 2: First order derivative Area under Curve spectrum of Ampicillin Trihydrate in Methanol (25 μ g/ml)

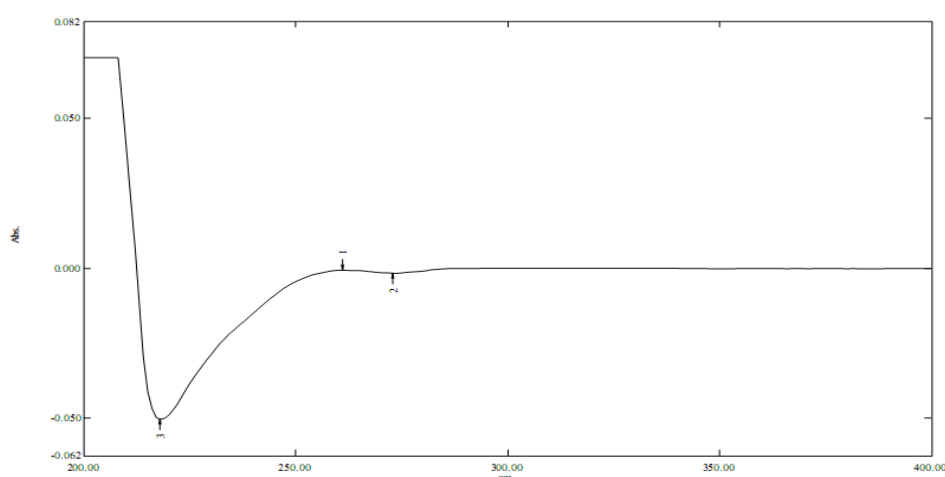


Fig. 3: First order derivative spectrum of Ampicillin Trihydrate in Methanol (25 μ g/ml).

2.5.2 Calibration curve for Ampicillin Trihydrate

The dilutions were made from Standard Stock solution to get concentration of 5, 10, 15, 20, and 25 μ g/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 224-231 nm. The calibration curve was plotted between areas under curve values against concentration.

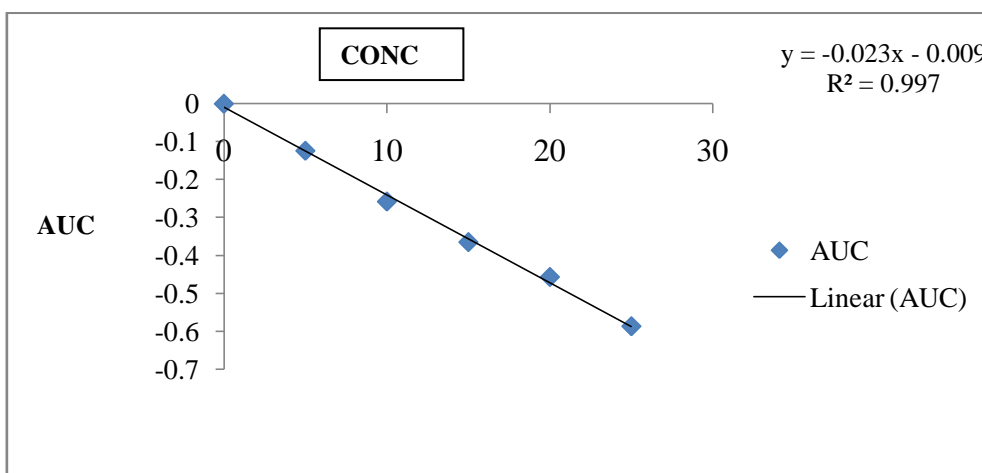


Fig. 4: Linearity of Ampicillin Trihydrate

2.5.3 Assay of tablet formulation

Twenty tablets each containing 500mg of Ampicillin Trihydrate were weighed crushed to powder and average weight was calculated. Powder equivalent to 10 mg of Ampicillin Trihydrate was transferred in 100 ml of volumetric flask. A 50 ml of Methanol was added and sonicated for 15 minutes.

Then solution was further diluted up to the mark with Methanol. 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 Methanol as blank. This procedure was repeated three times.

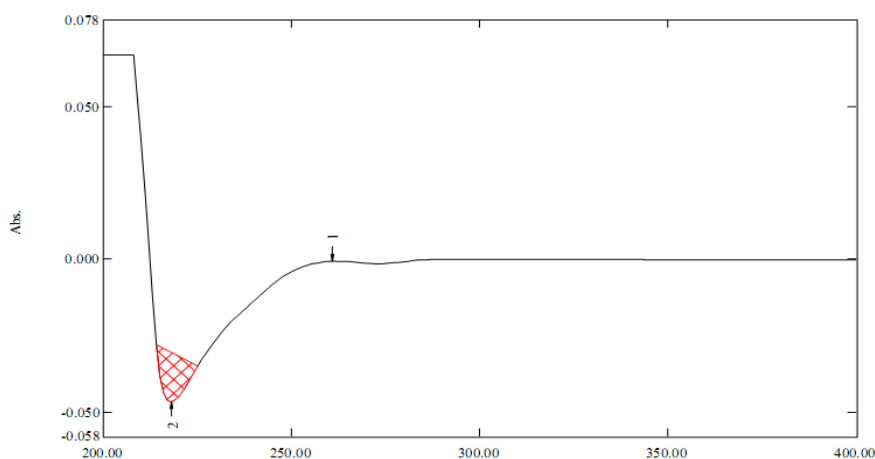


Fig. 5: First order derivative Area under Curve spectrum of Ampicillin Trihydrate of dosage form in Methanol (25µg/ml)

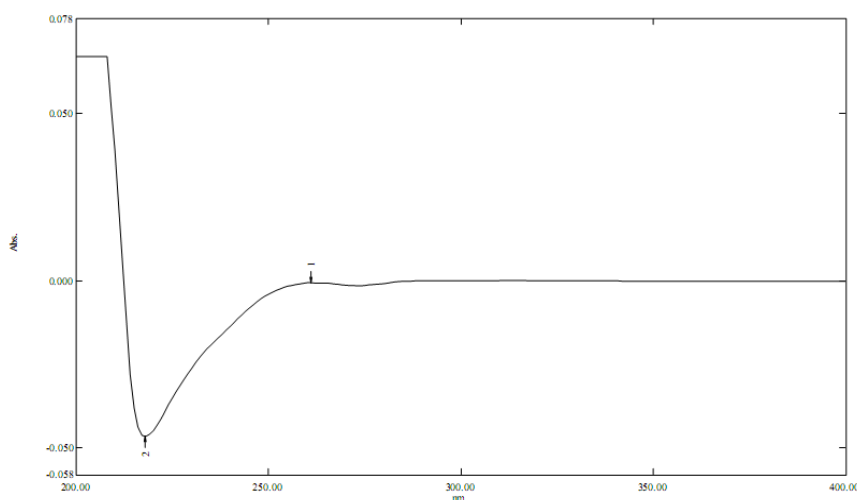


Fig. 6: First order derivative spectrum of Ampicillin Trihydrate of dosage form in Methanol (25µg/ml)

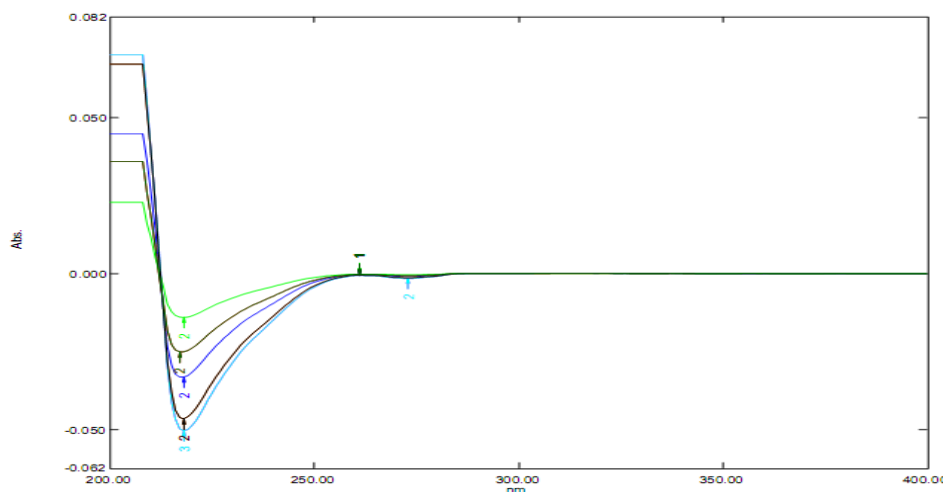


Fig. 7: First order derivative overlay of Ampicillin Trihydrate at diff. Concentration

Table 1: Assay of tablet dosage form:-

Sr. No.	Sample Solution Concentration (µg/ml)	Amount found (%)	Mean % found*	%RSD*
1	25	99.29		
2	25	100.43	99.27	0.0109
3	25	98.09		

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

3. Method Validation

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.

3.1 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 224-231 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 Ampicillin Trihydrate

Accuracy level	Sample conc (µg/ml)	Std. conc	Total amount. Added (µg/ml)	% Recovery	Mean % Recovery	% RSD
80	25	12	22	98.36		
100	25	15	25	102.87	100.68	0.0481
120	25	18	28	100.82		

3.2 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)	0.0049	0.0051
%RSD	0.7581	0.7958

3.3 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 Ampicillin Trihydrate was found to be 0.59µg/ml & 1.47µg/ml respectively.

Table 4: Summary of validation parameters

Parameter	Result
λ range	224-231
Regression Equation (y=mx+c)	Y=-0.023x - 0.009
Linearity range	5-25 $\mu\text{g/ml}$
Slope	0.023
Intercept	0.009
Correlation coefficient (R^2)	0.997
Limit of Detection (LOD) $\mu\text{g/ml}$	0.59
Limit of Quantitation (LOQ) $\mu\text{g/ml}$	1.47
Accuracy (Mean % Recovery)	100.68
Precision (%RSD)	0.0481

4. Results and Discussion

The UV visible spectroscopic method for the Ampicillin Trihydrate 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 $\mu\text{g/ml}$ and the correlation coefficient value of 0.997 indicates that developed method was linear. For Precision the percent relative standard deviation (% RSD) was found to be 0.0481 while, intra-day and inter-day precision results in terms of percent relative standard deviation values were found to be 0.7581 and 0.7958 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 0.59 $\mu\text{g/ml}$ & 1.47 $\mu\text{g/ml}$ 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 Ampicillin Trihydrate in bulk and pharmaceutical formulations.

5. Conclusion

The UV spectroscopic AUC method for the analysis of Ampicillin Trihydrate 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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