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Research Article

ZERO ORDER AND AREA UNDER CURVE SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF AMOXICILLIN TRIHYDRATE IN PHARMACEUTICAL FORMULATION.

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ABSTRACT

Objective:A simple, accurate, precise and specific zero order and area under curve spectrophotometric methods has been developed for determination of Amoxicillin Trihydrate in its tablet dosage form by using methanol as a solvent.

Methods: Derivative Spectrophotometric Methods: - The amplitudes in the zero order derivative of the resultant spectra at 229 nm was selected to find out Amoxicillin Trihydrate in its tablet dosage form by using methanol as a solvent.

Area under curve (Area calculation): -The proposed area under curve method involves measurement of area at selected wavelength ranges. Two wavelength ranges were selected 224-232 nm for estimation of Amoxicillin Trihydrate.

Result & Discussion: -The linearity was found to be 5-25 µg/ml for Amoxicillin Trihydrate. The mean % recoveries were found to be 100.33% and 99.42% of zero order derivative and area under curve method of Amoxicillin Trihydrate. For Repeatability, Intraday precision, Interday precision, % RSD were found to be 0.0036, 0.0028 and 0.5987, 0.4188 for zero order and 0.0019, 0.0031 and 1.6357, 1.7159 for area under curve method respectively. Limit of Detection and Limit of Quantitation were found to be 0.9142µg/ml and 2.8128µg/ml for zero order and 0.5798µg/ml and 1.3153µg/ml for area under curve method respectively. Assay results of market formulation were found to be 100.84% for zero order and 99.65% area under curve method respectively. The proposed method has been validated as per ICH guidelines and successfully applied to the estimation of Amoxicillin Trihydrate in its Tablet dosage form.

Conclusion: - The developed methods can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

Keywords: - Amoxicillin Trihydrate, UV visible spectrophotometry, AUC, Method, Validation.

INTRODUCTION

Amoxicillin Trihydrate is chemically (2S,5R,6R)-6-[(2R)-2-amino-2-(4hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2 carboxylic acid [1,2]. Amoxicillin Trihydrate is amino Penicillin with spectrum similar to that of Ampicillin [3]. Amoxicillin Trihydrate is used in the treatment of a number of infections, including acute otitis media, streptococcal pharyngitis, pneumonia, skin infections, urinary tract infections, Salmonella infections, Lyme disease, and chlamydia infections [4,5]. It is also used to prevent bacterial endocarditis in high-risk people having dental work done, to prevent Streptococcus pneumoniae and other encapsulated bacterial infections in those without spleens, such as people not appeared to have changed the rates of infection for infectious endocarditis [6,7]. In our Literature survey reveals that for Amoxycillin Trihydrate Spectrophotometric [8,9] methods and HPLC [10] methods have been reported for its determination in commercial formulation. To our notice, no UV- spectrophotometric method using zero order and area under curve (AUC) for determination of Amoxycillin Trihydrate in its formulations. The United Kingdom recommends against its use for infectious endocarditis prophylaxis. These recommendations have reported for the determination of Amoxicillin Trihydrate in bulk and tablets. Hence an attempt has been made to develop new zero order and area under curve spectrophotometric methods for estimation of Amoxicillin Trihydrate in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

Molecular formula: $C_{16}H_{19}N_3O_5S$

Molecular weight: 365.404 g/mol

MATERIALS AND METHODS

Apparatus and instrumentation

A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single

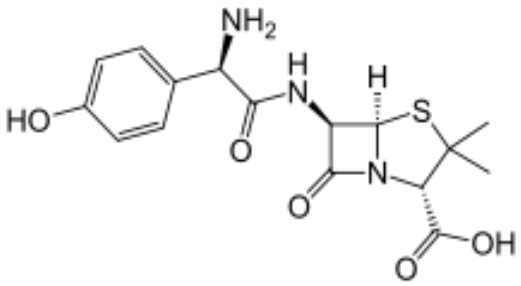

Fig. 1: Chemical Structure of Amoxicillin Trihydrate.

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.

MaterialsReference standard of Amoxicillin Trihydrate API was supplied as gift sample by Lupin Laboratory Park Aurangabad, Maharashtra, India. Tablet sample with label claim 500 mg per tablet were purchased from local market Andhalgaon, Tal:-Mangalwedha, Dist:-Solapur, Maharashtra, India.

METHOD DEVELOPMENT

Preparation of Standard and Sample Solutions

Stock solution of 10µg/ml of Amoxicillin Trihydrate was prepared in Methanol, for zero order and area under the 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 µg/ml with Methanol for zero order and area under the curve spectrophotometric methods. Methanol was used as a blank solution.

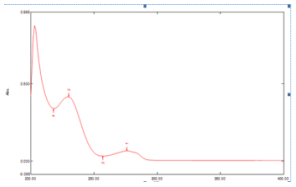

Fig. 2 Zero order derivative spectrum of Amoxicillin Trihydrate in Methanol (10µg/ml).

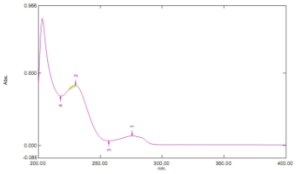

Fig. 3 UV AUC spectrum of Amoxicillin Trihydrate in Methanol (10µg/ml).

Area under curve (Area calculation)

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ1 and λ2 representing start and end point of curve region. The area under curve between λ1 and λ2 was calculated using UV probe software. In this study area was integrated between wavelength ranges from 224 to 232 nm.

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

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 [11].

Assay Procedure

Twenty tablets each containing 500 mg of Amoxicillin Trihydrate were weighed crushed to powder and average weight was calculated. Powder equivalent to 10mg of Amoxicillin 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 15 µg/mL solution with Methanol subjected for UV analysis using Methanol as blank. Appropriate dilutions were made with methanol from stock solution for both zero order and area under the curve spectrophotometric methods.

Table 1: Assay of tablet dosage form.

Sr.No.	Sample Solution Concentration (µg/ml)	Amount found (%)* Zero derivative	Amount found (%)* Auc	Mean % Found zero derivative	Mean % Found Auc	%RSD zero derivative	%RSD Auc
1	15	102.37	99.35				
2	15	98.60	98.55	100.33	99.42	0.3698	0.4789
3	15	100.02	100.38				

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

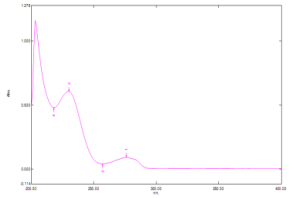

Fig. 4 Zero order derivative spectrum of Amoxicillin Trihydrate in Methanol dosage form (15µg/ml).

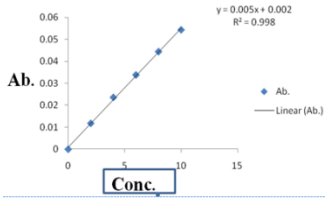

Fig.5 Linearity of Amoxicillin Trihydrate by Absorbance

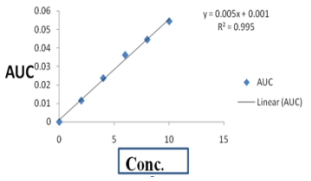

Fig.6 Linearity of Amoxicillin Trihydrate by AUC.

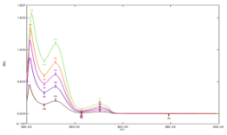

Fig. 7 Zero order derivative overlay of Amoxicillin Trihydrate at diff. Concentration.

RESULTS AND DISCUSSION

The zero order and area under the curve spectra for Amoxicillin Trihydrate were recorded at the wavelength of 229nm and 224-232nm respectively.

Linearity and Range

Under the experimental conditions described, the graph obtained for zero order and area under the curve spectra showed linear relationship. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were y = 0.005x+0.002 (r^2=0.998) at 229 nm for zero order derivative spectrophotometry and y= 0.005x+0.001 (r^2=0.995) at 224-232 nm for area under the curve spectrophotometry. The range was found to be 5, 10,15,20,25 µg/ml for both zero order and area under the curve spectrophotometric methods [12, 13].

Table 2: Stastical data for the calibration graphs for determination of Amoxicillin Trihydrate by Proposed methods.

Parameters	Zero order derivative	Area Under the Curve
Linearity range (µg/ml)*	5-25	5-25
r^2± S.D*	0.998	0.995

Accuracy

To study the accuracy of the proposed methods and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 15µg/ml standard solution. For Area under curve (AUC) was measured in wavelength range 224-232 nm and for Zero order derivative at 229 nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level [12, 13].

Precision

To determine the precision of the method Amoxicillin Trihydrate solutions at a concentration of 10µg/ml were analysed each three times for both zero order and area under the curvespectrophotometric methods. Solutions for the standard curves were prepared fresh everyday [12, 13].

Table 3: Accuracy results for Amoxicillin Trihydrate.

Accuracy level	Sample conc (µg/)	Std. conc	Total amnt. Added (µg/m)	%Recovery zero derivatie	% Recovery Auc*	Mean of Zero derivative*	Mean of Auc	% RSD Zero derivative	% RSD Auc
80	15	12	27	101.17	99.37				
100	15	15	30	98.63	100.28	100.84	99.65	0.429	0.718
120	15	18	33	102.74	99.31				

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

Table 4:- Results of Intra and Inter Day Precision:-

Parameters	Intra Day Precision		Inter Day Precision	
	S.D*	% RSD*	S.D*	% RSD*
Zero derivative	0.0036	0.5987	0.0028	0.4188
Area under the curve	0.0019	1.6357	0.0031	1.7159

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3xσ/ S and LOQ = 10xσ/S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.9142µg/ml and 2.8128µg/ml respectively for zero order derivative and the LOD and LOQ were found to be 0.5798µg/ml &1.3153µg/ml for area under the curve methods respectively [12, 13].

Analysis of the Marketed Formulation:-

There was no interference from the excipients commonly present in the tablets. The drug content was found to be 100.51% and 98.64% zero order and area under the curve spectrophotometric methods respectively. It may therefore be inferred that degradation of Amoxicillin Trihydrate had not occurred in the marketed formulations that were analysed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of Amoxicillin Trihydrate pharmaceutical dosage form.

Table 5: Summary of validation parameters:-

Parameter	Zero derivative	AUC
λ range	200-400 nm	224-232nm
Regression Equation (y=mx+c)	Y=0.005x+0.002	Y=0.0123x+0.0119
Measured wavelength	229 nm	229nm
Linearity range	5-25µg/ml	5-25µg/ml
Slope	0.005	0.0123
Intercept	0.002	0.0119
Correlation coefficient (R^2)	0.998	0.995
Limit of Detection (LOD) µg/ml	0.9142	0.5798
Limit of Quantitation (LOQ) µg/ml	2.8128	1.3153
Accuracy (Mean % Recovery)	100.84	99.65
Precission (%RSD)	0.429	0.718

CONCLUSION

No UV or Area under Curve spectrophotometric methods have been described for the determination of Amoxicillin Trihydrate.

Therefore simple, fast and reliable derivative spectrophotometric methods were developed for the routine determination of Amoxicillin Trihydrate. The developed methods can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

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