

## Zero Order and Area Under Curve Spectrophotometric methods for determination of Oxcarbazepine in Pharmaceutical Formulation

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### Abstract

Simple, fast and reliable spectrophotometric methods were developed for determination of Oxcarbazepine in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in Distilled Water. The quantitative determination of the drug was carried out using the zero order derivative values measured at 256 nm and the area under the curve method values measured at 252-258 nm (n=2). Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Oxcarbazepine using 5-25 µg/ml ( $r^2=0.998$  and  $r^2=0.9986$ ) for zero order and area under the curve spectrophotometric method. All 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. Developed spectrophotometric methods in this study are simple, accurate, precise and sensitive to assay of Oxcarbazepine in tablets.

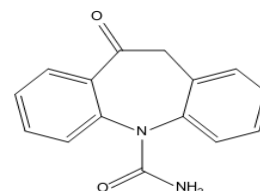
**Keywords:** Oxcarbazepine, UV visible spectrophotometry, AUC, Method Validation.

### 1. Introduction

Oxcarbazepine (OXC) is chemically known as, 10, 11-dihydro-10-oxo-5H-dibenz (b,f) azepine-5-carboxamide[1] (Figure 1). OXC, is a keto analog of carbamazepine and is an anticonvulsant and mood stabilizing drug. OXC is known to exert antiepileptic activity by blockade of voltage-dependent sodium channels in the brain[2]. Literature survey revealed several analytical methods voltammetry[3], chiral liquid chromatography method with ultraviolet detection (LC-UV)[4,5], micellar electrokinetic chromatography[6], microemulsion electrokinetic chromatography[7], HPLC[8-20], capillary electrophoresis[21], GC-MS[22] and spectrophotometry[23-24] have been reported in bulk, pharmaceutical dosage form for determination of Cefadroxil. Hence an attempt has been made to develop new Zero Order and Area under Curve Spectrophotometric methods for estimation of

Oxcarbazepine in bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

**Fig. 1: Chemical structure of Oxcarbazepine.**



### 2. Materials and Methods

#### 2.1 Apparatus and instrumentation

A Shimadzu1800 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.

## 2.2 Materials

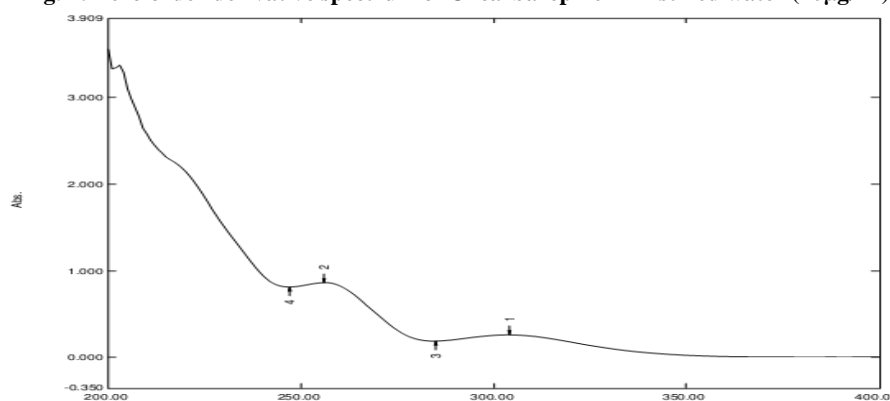
Reference standard of Oxcarbazepine API was supplied as gift sample by Marksan Pharmaceutical Ltd., Verna, Goa. Tablet sample with label claim 200 mg per tablet were purchased from local market Pune, Maharashtra, India.

## 2.3 Method development

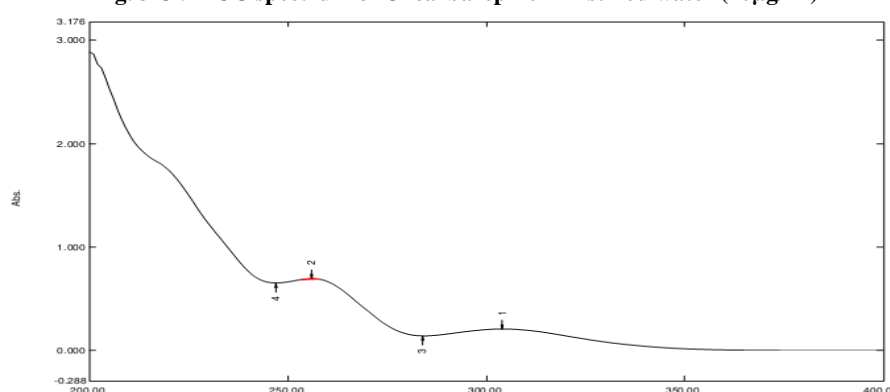
### 2.3.1 Preparation of Standard and Sample Solutions:-

Stock solution of 20µg/ml of Oxcarbazepine was prepared in Distilled Water, for zero order and area under the curve spectrophotometric analysis. The standard solutions were prepared by dilution of the stock solution with Distilled water in a concentration range of 5, 10, 15, 20, and 25µg/ml with Distilled water for zero order and area under the curve spectrophotometric methods. Distilled water was used as a blank solution.

**Fig. 2: Zero order derivative spectrum of Oxcarbazepine in Distilled water (20µg/ml)**



**Fig. 3 UV AUC spectrum of Oxcarbazepine in Distilled water (20µg/ml)**



## 2.4 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  $\lambda_1$  and  $\lambda_2$  representing start and end point of curve region. The area under curve between  $\lambda_1$  and  $\lambda_2$  was calculated using UV probe software. In this study area was integrated between wavelength ranges from 252 to 258 nm.

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

Where,  $\alpha$  is area of portion bounded by curve data and a straight line connecting the start and end point,  $\beta$  is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis,  $\lambda_1$  and  $\lambda_2$  are wavelength range start and end point of curve region[24].

## 2.5 Assay Procedure

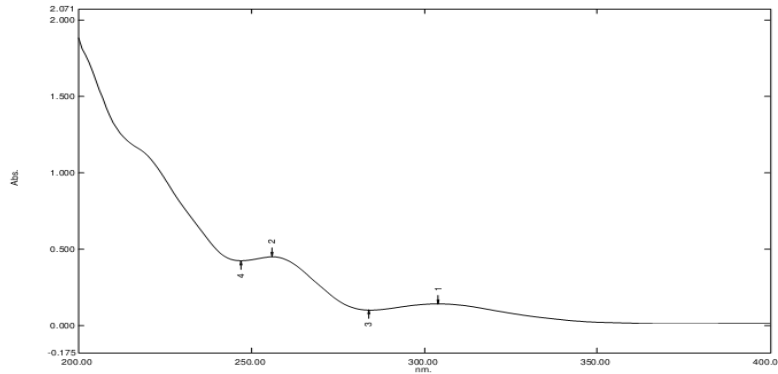
Twenty tablets each containing 200 mg of Oxcarbazepine were weighed crushed to powder and average weight was calculated. Powder equivalent to 10 mg of Oxcarbazepine was transferred in 100 ml of volumetric flask. 50 ml of distilled water was added and sonicated for 15 minutes. Then solution was further diluted up to the mark with distilled water. The solution was filtered using Whatmann filter paper no. 41; first 5 ml of filtrate was discarded. This solution was further diluted to obtain 15µg/mL solution with water subjected for UV analysis using distilled water as blank. Appropriate dilutions were made with Distilled water 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	98.91	97.27				
2	15	99.89	99.21	99.50	98.07	0.5242	1.0302
3	15	99.71	97.75				

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

**Fig. 4 Zero order derivative spectrum of Oxcarbazepine dosage form (15µg/ml).**



**3. Results and Discussion**

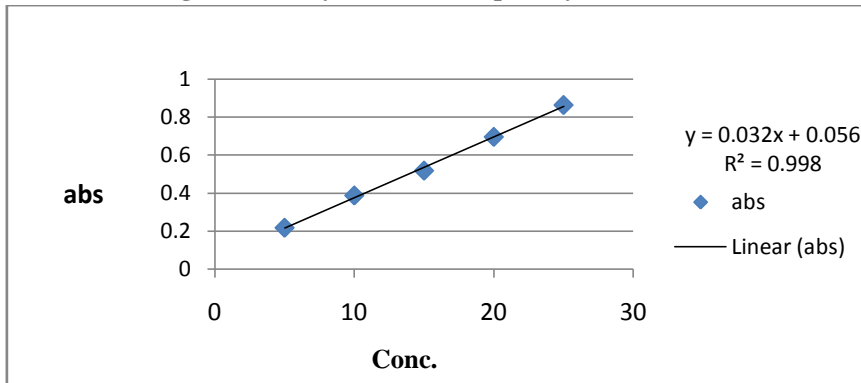
The zero order and area under the curve spectra for Oxcarbazepine were recorded at the wavelength of 256nm and 252-258 nm respectively [Fig. 2 and 3].

**3.1 Linearity and Range**

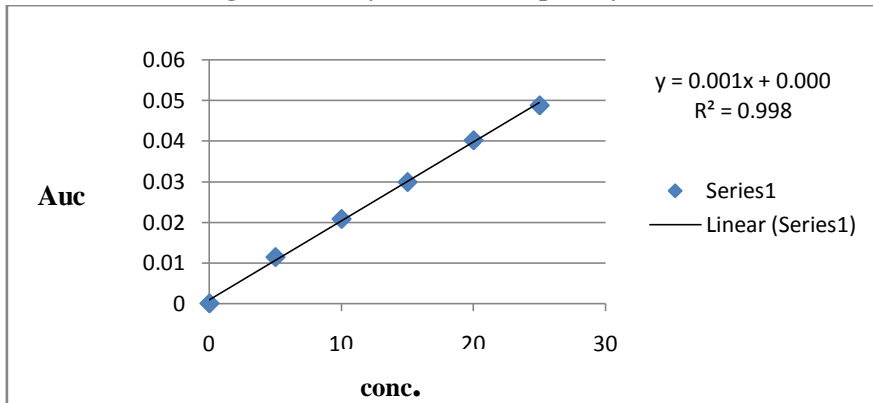
Under the experimental conditions described, the graph obtained for zero order and area under the curve spectra showed linear relationship.

Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were  $y=0.0321x+0.0565$  ( $r^2=0.9980$ ) at 256 nm for zero order derivative spectrophotometry and  $y=0.0019x+0.0009$  ( $r^2=0.9986$ ) at 252-258 nm for area under the curve spectrophotometry. The range was found to be 5-25µg/ml for both zero order and area under the curve spectrophotometric methods.

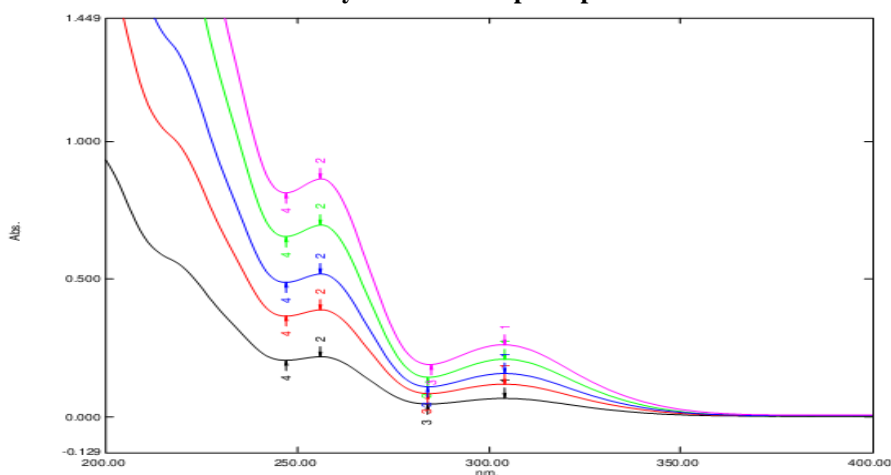
**Fig.5: Linearity of Oxcarbazepine by Absorbance**



**Fig.6: Linearity of Oxcarbazepine by AUC**



**Fig. 7: Zero order derivative overlay of Oxcarbazepine spectra at different Concentration.**



**Table 2: Statistical data for the calibration graphs for determination of Oxcarbazepine by Proposed methods**

Parameters	Zero order derivative	Area Under the Curve
Linearity range (µg/ml)*	5-25	5-25
r <sup>2</sup>	0.9980	0.9986

**3.2 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 20µg/ml

standard solution. For Area under curve (AUC) was measured in wavelength range 252-258 nm and For Zero order derivative at 256 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 3: Accuracy results for Oxcarbazepine.**

Accuracy level	Sample conc (µg/m)	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	97.13	99.17				
100	15	15	30	99.14	99.18	98.72	100.29	1.445	1.937
120	15	18	33	99.89	102.54				

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

**3.3 Precision**

To determine the precision of the method, Oxcarbazepine solutions at a concentration of 20µg/ml were analysed each three times for both zero

order and area under the curve spectrophotometric methods. Solutions for the standard curves were prepared fresh everyday (Table II).

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

Parameters	Intra Day Precision		Inter Day Precision	
	S.D*	% RSD*	S.D*	% RSD*
Zero derivative	0.0076	1.1090	0.0051	0.7193
Area under the curve	0.0408	0.7063	0.0486	0.7784

**3.4 Sensitivity**

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations  $LOD = 3x\sigma / S$  and  $LOQ = 10x\sigma / S$ , where  $\sigma$  is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 1.3381µg/ml and 4.0550µg/ml respectively for zero order derivative and The LOD and LOQ were found to be 0.9676µg/ml & 2.1140µg/ml for area under the curve methods respectively.

**3.5 Analysis of the Marketed Formulation**

There was no interference from the excipients commonly present in the tablets. The drug content was found to be 100.006% and 100.01% zero order and area under the curve spectrophotometric methods respectively. It may therefore be inferred that degradation of Oxcarbazepine 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 Oxcarbazepine in pharmaceutical dosage form (Table 4).

**Table 5: Summary of validation parameters**

Parameter	Zero derivative	AUC
$\lambda$ range	200-400 nm	252-258nm
Regression Equation (y=mx+c)	Y=0.0321x+0.0565	Y=0.0019x+0.0009
Measured wavelength	256 nm	256nm
Linearity range	5-25 $\mu\text{g/ml}$	5-25 $\mu\text{g/ml}$
Slope	0.0321	0.0019
Intercept	0.0565	0.0009
Correlation coefficient ( $R^2$ )	0.998	0.9986
Limit of Detection (LOD) $\mu\text{g/ml}$	1.3381	0.9676
Limit of Quantitation (LOQ) $\mu\text{g/ml}$	4.0550	2.1140
Accuracy (Mean % Recovery)	98.72	100.29
Precision (%RSD)	1.1090	0.7063

#### 4. Conclusion

No UV or Area under Curve spectrophotometric methods exists for the determination of Oxcarbazepine. Therefore simple, fast and reliable derivative spectrophotometric methods were developed for the routine determination of Oxcarbazepine. The developed methods can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

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