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Estimation of Carbamazepine in Bulk and Pharmaceutical Dosage Form By Zero Order and Area under the Curve UV Spectrophotometric Methods

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Abstract*—The aim of the present research was broadly focused on the estimation of carbamazepine in bulk and pharmaceutical dosage form by using two UV –Spectrophotometric methods namely, Zero order UV spectrophotometry (Method -1) and Area under the curve UV spectrophotometry (Method -2). The Zero order UV Spectrophotometric method was based on the measurement drug absorbance at wave length of 284 nm, which was its wavelength of maximum absorbance .The Area under the curve method, was based on the calculation of area occupied by the UV absorbance curve between 278-290 nm. The solvent employed for both methods was 50% v/v ethanol. In the estimation of Carbamazepine, both the methods showed linearity in the range of 2-12µg/ml. The correlation co-efficient was ≥ 0.999. The precision for both the methods was ≤2% RSD. The accuracy was performed by using percentage recovery studies of standard drug spiked at 50,100 and 150% of the test concentration and the values obtained were within the limits. The developed methods were applied for the assay of the drug in its respective dosage forms. The assay of pharmaceuticals dosage form was found to be within limits. All the results were satisfactory, the developed methods can be routinely used for the analysis of the drugs in both bulk and dosage forms*.

***Keywords*—** *Area under the curve, Assay, Carbamazepine, Validation, Zero order UV Spectrophotometry*.

I. INTRODUCTION

Carbamazepine is a sodium channel blocker. It is widely used for the treatment of Epilepsy. It has additional action in the treatment of seizures, which may be a side effect of other drugs used in surgery. It binds preferentially to voltage-gated sodium channels in their inactive conformation, which prevents repetitive and sustained firing of an action potential. Carbamazepine has effects on serotonin systems but the relevance to its antiseizure effects is uncertain. There is evidence that it is a serotonin releasing agent and possibly even a serotonin reuptake inhibitor[1-2]. The chemical structure of Carbamazepine was shown in figure 1 [1-2].

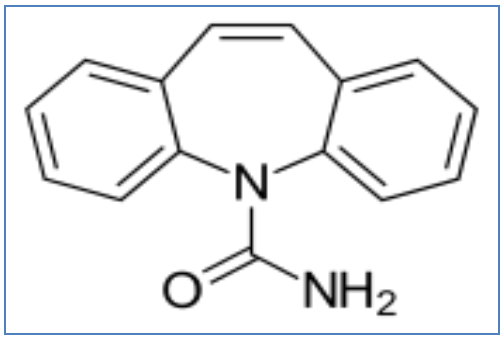

Fig. 1. Chemical structure of Carbamazepine

Extensive survey of literature [3-12] on Carbamazepine revealed that the reported methods were mostly aimed for the clinical studies, pharmacokinetic evaluation, formulation and dissolution studies of the drug and only few analytical methods based on HPLC were reported for the quantification of Carbamazepine. Thus, there is a need to develop simple and economical UV methods for the quantification of Carbamazepine in bulk and dosage forms according to ICH guidelines [15].

The aim of the present research was broadly focused on the estimation of carbamazepine in bulk and formulation by using two UV –Spectrophotometric methods namely, Zero order UV spectrophotometry (method -1) and Area under the curve UV spectrophotometry (method -2).

II. MATERIALS AND METHODS

Instruments Used:

UV Visible double beam Spectrophotometer (UV 1800) of Shimadzu, Japan was used in the study connected to system and operated with UV probe as data handling system.

Materials Used:

The working standard carbamazepine was a gift sample form Novartis Laboratories. Ethanol analytical reagent (AR) grade was procured from E. Merck Ltd., Mumbai. Double distilled water was prepared using Milli Q system. Formulation of carbamazepine (Tegrital®200) was purchased from local pharmacy.

Methods:

Zero order UV-Spectrophotometric method development:

Different Solvents like Water, Ethanol, 50% Ethanol, were employed for recording of the UV spectrum and for the optimization of the method.

Preparation of Stock solution

Carbamazepine pure 25 mg was weighed and transferred to a 25 ml volumetric flask and dissolved in 50% ethanol. It was dissolved properly and diluted up to the mark with diluent to obtain final concentration of 1000 µg/ml. optimized solution was prepared from the stock solution with distilled water, which was used as working standard.

Calibration curve for Carbamazepine

From the standard stock solution of Carbamazepine appropriate aliquots were pipetted out in to 10 ml volumetric flasks and dilutions were made with distilled water to obtain working standard solutions of concentrations from 2-12 µg/ml. Absorbance for these solutions were measured at 284 nm. The standard solution analytical concentration range was found to be 2-12µg/ml and those values were reported in Table I. The regression equation and correlation coefficient was determined and are presented in Table I.

Area under the Curve (AUC)-UV Spectrophotometric Method:

The principle for Area under curve method is "the area under two points on the spectra is directly proportional to the concentration of the compound of Interest". AUC is particularly suitable for the compounds where there is no sharp peak or broad spectra were obtained, AUC method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range was selected based on repeated observations to get the linearity between area under curve and concentration. Validation of the developed spectrophotometric methods

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of the analyte in the sample.

Range

Range is the difference between upper concentration and lower concentration. The results obtained are within the range.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements from multiple sampling of the same homogeneous sample under prescribed conditions. The concentration of 3µg/ml, 6µg/ml, and 9µg/ml were selected for precision. They are prepared by taking 0.03ml, 0.06ml, 0.09 ml from stock solution-1 respectively into the 10ml volumetric flask and made up to the mark with water and was analyzed by using UV spectrophotometer.

Accuracy

To determine the accuracy of the proposed method different levels of drug concentrations were prepared from independent stock solutions and analyzed. To provide an additional support to the accuracy of the developed assay method, a standard addition method was employed, which involved the addition of different concentrations of pure drug to a known pre-analyzed dilution of the pure drug and the total concentration was determined using the proposed method. The % recovery levels of the added sample drug were, calculated. The recovery studies were performed at different levels like 50%, 100% and 150% of the target concentration (6 mcg/ml). The amount of standard drug recovered from the spiked test samples was calculated from the absorbance values.

Assay Procedure

1mg of pharmaceutical dosage form of Carbamazepine was taken which consists of 10mg/ml (10000µg/ml) concentration; this solution was diluted to 10ml to give the concentration of 1000µg/ml (stock solution-1). Stock solution-2 was prepared by taking 1ml solution from stock solution-1 in to the 10ml volumetric flask and made up to the mark with water to produce 100µg/ml.

Assay Calculation

The quantity of Carbamazepine was calculated from calibration curve using absorbance value of test formulation.

III. Results and Discussion

Validation of Zero order UV-Spectrophotometric Method:

Carbamazepine in distilled water gave a single distinct peak with good absorbance in the recorded UV spectrum, so it was employed as the solvent. The wavelength of maximum absorbance (λ_{max}) for the bulk drug was found to be 284nm. The UV absorbance spectrum was shown in figure 2.

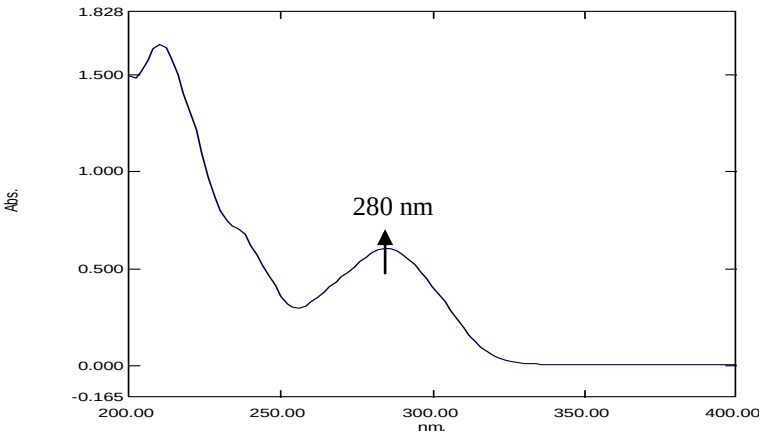

Fig. 2. Zero order spectra of Carbamazepine showing absorbance at 284nm

Linearity and Range:

Carbamazepine showed good linearity in the range of 2-12µg/ml. The correlation coefficient was found to be 0.999. The linearity data was shown in table I and figure 2, 3 & 4.

Precision (Repeatability):

Both intra-day and inter-day precision was within the acceptable limit with a % RSD less than 2%. So, the developed method was more precise and repeatable.

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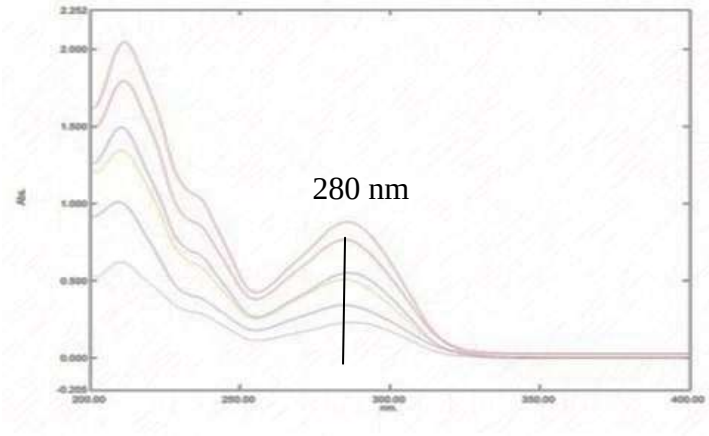

Fig. 3. Overlay spectra showing linearity of Carbamazepine

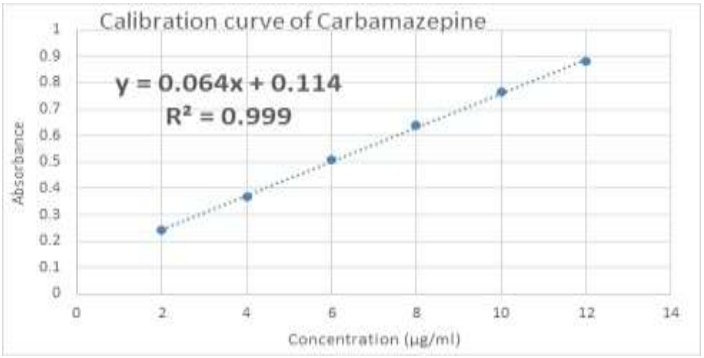

Fig. 4. Linearity graph of Carbamazepine UV-Spectrophotometry

Accuracy:

The recovery studies with standard addition method at 50%, 100% and 150% levels of the test concentration showed good results with a mean recovery of 99.85 %. The developed method was accurate. The results were shown in table II.

Limit of Detection and Quantification (LOD & LOQ):

The LOD was found to be 0.16 µg/ml and LOQ was 0.49 µg/ml.

Assay:

The assay of Carbamazepine was calculated by using calibration curve method and was found to be 100.393 %. The consolidated results of the validation parameters of the developed method were shown in table V.

Validation of Area under the Curve UV-Spectrophotometric Method: For the selection of analytical wavelength, 6µg/ml solution of was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. From the spectra of drug, area under the curve in the range of 278-290nm was selected for the analysis as shown in the figure 5. The calibration curve was prepared in the concentration range of 2-12µg/ml at their respective AUC range. By using the calibration curve, the concentration of the sample solution can be determined.

Linearity and range: Carbamazepine showed good linearity in the range of 2-12µg/ml. The correlation coefficient was found to be 0.999. The linearity data was shown in table III and figure 6.

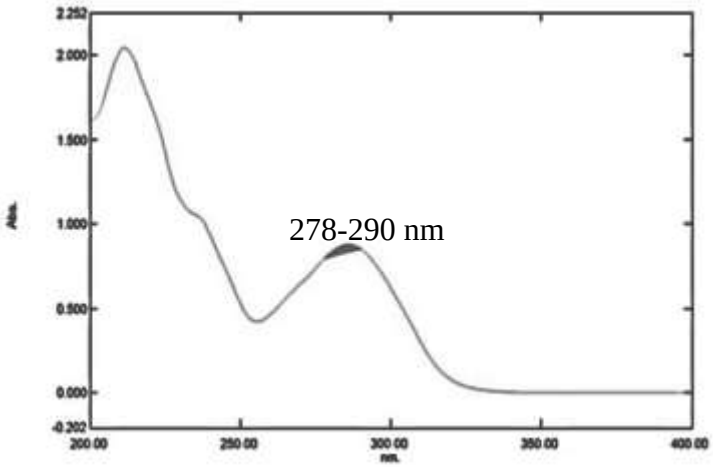

Fig. 5. Selection of wavelengths for Area under the curve method

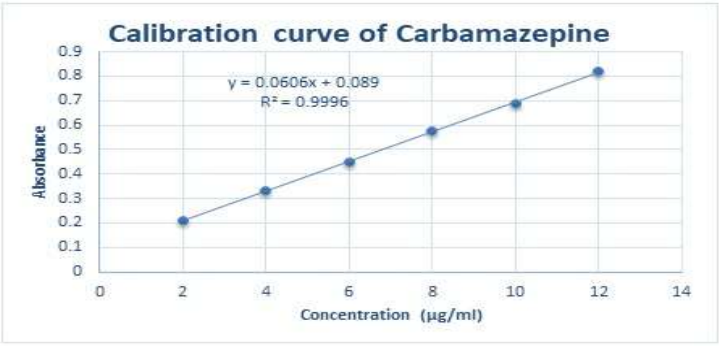

Fig. 6. Linearity graph of Carbamazepine -AUC Method

Precision (Repeatability): Both intra-day and inter-day precision was within the acceptable limit with a % RSD less than 2%. So, the developed method was more precise and repeatable.

Accuracy: The recovery studies with standard addition method at 50%, 100% and 150% levels of the test concentration showed good results with a mean recovery of 99.45 %. The developed method was accurate. The results were shown in table IV.

Assay: The assay of Carbamazepine was calculated by using calibration curve method and was found to be 101.33 %. The consolidated results of the validation parameters of the developed method were shown in table V.

The overall summary of optical characteristics and other validation parameters of zero order and Area under curve (AUC) spectroscopic methods were shown in table V.

TABLE I. Linearity of Carbamazepine UV-Spectrophotometry

S.No	Concentration (µg/ml)	Absorbance ± Standard Deviation
1	2	0.241 ± 0.012
2	4	0.366 ±0.010
3	6	0.510 ± 0.097
4	8	0.638 ± 0.016
5	10	0.766 ± 0.015
6	12	0.880 ± 0.019
Equation for regression liney = 0.064x - 0.0114		**Correlation coefficient ≥0.999**

TABLE II. % Recovery for Zero order UV-Spectrophotometry

Recovery range	Test Concentration (µg/ml)	Amount of standard concentration spiked (µg/ml)	Amount of sample concentration found (µg/ml)	% Recovery
50	6	3	102.109	101.457
			101.348	
			100.935	
100		6	99.565	99.81
			100.348	
			99.761	
150		9	99.109	98.413
			97.135	
			98.196	

TABLE III. Linearity of Carbamazepine-AUC Method

S.No	Concentration (µg/ml)	Absorbance ± Standard Deviation
1	2	0.210 ± 0.002
2	4	0.334 ± 0.011
3	6	0.449 ± 0.016
4	8	0.579 ±0.013
5	10	0.689 ±0.011
6	12	0.82 ±0.013
Equation for regression line y = 0.060x -0.089		Correlation coefficient (R2) =0.999

TABLE IV. % Recovery-AUC Method

Recovery range	Test Concentration (µg/ml)	Amount of standard concentration spiked (µg/ml)	Amount of sample concentration found (µg/ml)	% Recovery
50%	6	3	100.55	99.67
			98.80	
			99.67	
100%		6	99.46	98.51
			97.72	
			98.37	
150%		9	99.38	100.16
			103.01	
			98.08	

TABLE V. Validation parameters of carbamazepine in Zero order spectroscopy and Area under curve methods

Validation Parameters	Results for Carbmazepine	
	Zero order spectroscopy	Area under curve method
Absorption Maxima (nm)	284	278-290 nm
Beer's-Lambert'srange(µg/ml)	2-12	2-12
Regression equation (y)*	Y = 0.064x+0.114	Y = 0.060x+0.089
Slope (b)	0.064	0.060
Intercept (a)	0.114	0.089
Correlation coefficient (r^2)	0.999	0.999
Intraday precision (% RSD)**	0.61	0.91
Interday precision (% RSD)**	0.89	1.18
Accuracy (% mean recovery)	99.85	99.44
Assay of tablets (%Purity)	100.393	101.33

IV. CONCLUSION

The proposed study describes a novel UV spectrophotometric method for the estimation of carbamazepine in bulk and pharmaceutical dosage form using suitable diluent. The method was validated and found to be simple, rapid, selective, accurate, precise and robust when compared to other methods. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. The method is also cost effective with respect to solvent consumption. Therefore, the proposed method can be used for routine analysis of carbamazepine in its dosage form.

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