UV SPECTROPHOTOMETRIC ESTIMATION OF CARBAMAZEPINE IN BULK AND TABLET DOSAGE FORM USING AREA UNDER CURVE METHOD

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ABSTRACT

The aim of present work was to develop an accurate, precise, reproducible and economical UV spectrophotometric Area Under Curve method for estimation of Carbamazepine. This Area Under Curve of UV spectrum between 275 to 295 nm was validated as per ICH guideline Q2 (R1). The method has followed linearity in the range of 2-12 μg/ml. The value of correlation coefficient was 0.999. Satisfactory values of Percent relative standard deviation for the intra-day and inter-day precision indicated that method is precise. Results of the recovery studies (99.67% to 101.18%) showed accuracy of the method. LOD and LOQ were calculated as 0.2984 μg/ml and 0.9042 μg/ml, respectively. The developed method can be used for routine estimation of Carbamazepine in bulk and tablet dosage forms.

KEYWORDS: Carbamazepine, UV spectrophotometry, Area Under Curve, validation.

INTRODUCTION

Carbamazepine is 5H-dibenz [b,f]azepine-5-carboxamide. Carbamazepine controls the convulsions (fits) suffered by people with epilepsy by reducing the activity of the central nervous system. Carbamazepine is also used in trigeminal neuralgia (severe burning or stabbing pains in the face), and occasionally as part of the treatment for bipolar disorder or manic depression. [1] Carbamazepine is official in IP 2010. [2] Literature survey reveals that some methods have been developed for their determination by HPTLC, [3] HPLC [4-6] either alone or in combination, but no method was found for the carbamazepine by Area Under Curve UV spectrophotometry. This Area Under Curve method is useful for quantitative and...
qualitative analysis. The purpose of this work was to develop a simple, accurate, precise, reproducible and economical UV spectrophotometric Area Under Curve method for estimation of carbamazepine.

![Chemical Structure of Carbamazepine](image_url)

**Fig.1 Chemical Structure of Carbamazepine**

**MATERIALS AND METHODS**

**Apparatus and Instrumentation**

Shimadzu UV 1800 with UV ProbSoftware, was employed for this work. Single pan electronic balance (Shimadzu, ATY 224) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonicator (Spectra lab UCB 40, India). Calibrated volumetric glass wares (Borosil) were used in this study.

**Materials**

Commercially available tablets Tegretol®-100 containing 100 mg of Carbamazepine was procured from local pharmacy. AR-grade of Methanol (as a solvent) was purchased from Merck India Ltd., Mumbai, India.

**Method development**

**Preparation of standard solution**

The standard stock solution of Carbamazepine was prepared by transferring, accurately weighed, 100 mg of API to 100 ml of volumetric flask. The drug was dissolved with sonication in 50 ml of methanol and volume was made up to the mark by using methanol. The standard stock solution (1000 μg/ml) was further diluted with methanol to get the concentration of 10 μg/ml.

**Selection of wavelength range**

The standard solution of 10 μg/ml was scanned between 400 nm to 200 nm in UV spectrophotometer against methanol as blank after baseline correction. Wavelength range was selected around wavelength maxima (285 nm). Different working standards were prepared between 2-12 μg/ml. Various wavelength range were tried and final range between 275-295
nm was selected on the basis of linear relationship between area and corresponding concentration (Fig. 2).

Fig. 2 Area Under Curve for Carbamazepine

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 275 to 295 nm.

Preparation of calibration curve
Adequate dilutions were made from standard stock solution to obtain the concentration of 2, 4, 6, 8, 10 and 12 μg/ml respectively. These solutions were scanned from 400 to 200 nm and area under curve (AUC) was integrated in the range of 275-295 nm. The calibration curve was plotted between Area under curve against concentration (Fig. 3).
Method validation

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Precision, Accuracy, Limit of detection (LOD) & Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline.[7]

Linearity and Range

From standard stock solution adequate dilutions were prepared to obtain solution containing 2, 4, 6, 8, 10, 12, µg/ml of carbamazepine respectively. Solutions were scanned between 400 nm to 200 nm in spectrum mode. The area under curve was determined and calibration curve was plotted (Fig. 3). Regression equation and correlation coefficient were determined.

Method Precision

Repeatability

In precision study repeatedly, 6 samples of standard solution of carbamazepine (8µg/ml) were used. Area under curve of each of these solutions was measured in the range of 275-295 nm. Percentage relative standard deviation (% RSD) value was calculated Table 2.

Intermediate Precision (Reproducibility)

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days for 8 µg/ml standard solution of carbamazepine, respectively. This experiment was performed in triplicate. The percentage relative standard deviation (% RSD) values were calculated Table 2.
Table 2. Precision results for Carbamazepine

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µg/ml)</th>
<th>(Mean area ± SD)*</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine (n=6)</td>
<td>8</td>
<td>0.7569 ± 0.009292</td>
<td>1.2276</td>
</tr>
<tr>
<td>Intraday (n=3)</td>
<td>8</td>
<td>0.7564 ± 0.001229</td>
<td>0.1624</td>
</tr>
<tr>
<td>Interday (n=3)</td>
<td>8</td>
<td>0.7558 ± 0.0004</td>
<td>0.05292</td>
</tr>
</tbody>
</table>

* n=3

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations:

LOD = 3.3 \( \sigma \)/S and LOQ = 10 \( \sigma \)/S

Where \( \sigma \) is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability. Six sets of each known concentrations (2-12 µg/ml) were prepared and scanned. By using these spectra’s, regression equations were obtained. By taking average of slopes and standard deviation of y – intercept LOD and LOQ were calculated. The values of LOD and LOQ are given in Table 4.

Accuracy

The accuracy of the method was determined by recovery study carried out using standard addition method at three different levels. The resulting spiked sample solutions were assayed in triplicate. The accuracy was determined at 80 %, 100 % and 120 % levels of 10 µg/ml standard solution. Area under curve was measured in the range of 275 - 295 nm. The percentage recovery was calculated for each level. The results are tabulated in Table 3.

Table 3. Accuracy results for Carbamazepine.

<table>
<thead>
<tr>
<th>Accuracy level</th>
<th>Amount added (µg/ml)</th>
<th>Amount recovered (µg/ml)</th>
<th>% Recovery ± SD*</th>
<th>Mean Recovery (%)*</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>8</td>
<td>7.97</td>
<td>99.67 ± 0.0003</td>
<td>99.44</td>
<td>0.3279</td>
</tr>
<tr>
<td>100%</td>
<td>10</td>
<td>9.73</td>
<td>97.49 ± 0.0023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>12</td>
<td>12.13</td>
<td>101.18 ± 0.00045</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*n=3
Assay of tablet formulation
Twenty tablets were weighed and average weight was calculated. The tablet powder equivalent to 100mg of Carbamazepine was accurately weighed, transferred to a 100 ml of volumetric flask and diluted up to mark with methanol. The solution was filtered with Whatman filter paper No. 41 and the first 5 ml of filtrate was discarded and suitable aliquot was diluted to obtain 10μg/ml solution with same solvent and subjected for UV analysis. This procedure was repeated in triplicate Table 4.

RESULTS AND DISCUSSION
An attempt was made to develop a simple and specific AUC spectrophotometric method for the determination of Carbamazepine in tablet dosage form. The generated regression equation was \( \int_{275}^{295} A d\lambda = 0.096x + 0.003 \) \( (R^2 = 0.999) \). Where, \( \int_{275}^{295} A d\lambda \) is area under curve between 275 to 295 nm. ‘x’ is the concentration and R is correlation coefficient. The R² value as 0.999 indicates that developed method was linear. The proposed method was found to be precise as % R.S.D values for intraday as well interday precision were satisfactory. The drug at each of the 80%, 100% and 120% levels showed good recoveries. Hence, it can be said that this method was accurate. The LOD and LOQ were calculated as 0.2984 μg/ml and 0.9042 μg/ml, respectively. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for the routine analysis of the Carbamazepine in tablet dosage form. The validation parameters are summarized in Table 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength range</td>
<td>275-295 nm</td>
</tr>
<tr>
<td>Linearity range (μg/ml)</td>
<td>2–12</td>
</tr>
<tr>
<td>Regression Equation(y=mx+c)</td>
<td>( y = 0.096x + 0.003 )</td>
</tr>
<tr>
<td>Correlation Coefficient (R²)</td>
<td>( R^2 = 0.999 )</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
</tr>
<tr>
<td>Repeatability (*n=6)</td>
<td>1.2276</td>
</tr>
<tr>
<td>Intraday (*n=3)</td>
<td>0.1624</td>
</tr>
<tr>
<td>Interday (*n=3)</td>
<td>0.05292</td>
</tr>
<tr>
<td>Limit of Detection (LOD) μg/ml</td>
<td>0.2984</td>
</tr>
<tr>
<td>Limit of Quantitation (LOQ) μg/ml</td>
<td>0.9042</td>
</tr>
<tr>
<td>Accuracy (Mean % Recovery)</td>
<td>99.44%</td>
</tr>
<tr>
<td>Assay</td>
<td>96.26 ± 0.002723</td>
</tr>
</tbody>
</table>
CONCLUSION
It can be concluded from the results that the proposed method was accurate, precise and consistent for the determination of Carbamazepine in tablet dosage form. This method was validated as per ICH guideline Q2 (R1). Results suggest that this method can be used for routine estimation of Carbamazepine in bulk and tablet dosage forms.

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REFERENCES