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DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROSCOPIC METHOD FOR ESTIMATION OF CARBAMAZEPINE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A novel, simple, accurate, rapid, precise, reproducible and cost effective spectrophotometric method for the quantitative estimation of carbamazepine in a pharmaceutical formulation and validated according to the ICHQ2 (R1) guideline. Spiked carbamazepine solution was scanned over UV-visible range for its wavelength of maximum absorbance. Various calibration standards of carbamazepine were prepared and absorbance was recorded at wavelength of maximum absorbance. Calibration curve of concentration vs. absorbance was plotted and linearity and range was calculated. Various analytical method validation parameters viz. accuracy, precision, LOD, LOQ, and ruggedness were calculated using QC standards. The maximum wavelength of carbamazepine was found to be 246 nm. The drug obeyed beer lambert's law in the concentration range of 2-12 µg/ml with regression 0.9992 at 284 nm. The overall % recovery was found to be 99.06 to 99.60 % which reflects that the method was free from the interference of the impurities and other excipients used in the formulation. The low value of % RSD was indicative of accuracy and reproducibility of the method. The % RSD for inter-day and intra-day precision was found to be of 0.56 to 1.82 & 0.36 to 0.59 respectively which is<2% hence proved that method is precise. The results of analysis have been validated as per International Conference on Harmonization (ICH) guidelines. The developed method can be adopted in routine analysis of carbamazepine in tablet dosage form as well bulk dosage form.

KEYWORDS: UV- visible spectrometry, Carbamazepine, Validation.

INTRODUCTION

Carbamazepine, 5H dibenzo (b, f) azepine-5-carboxamide (fig. 1) is an antiepileptic drug and it is the drug of choice for treatment of grand mal and psychomotor epilepsy. It is considered to be one of the most vital drugs for the relief of pain associated with trigeminal neuralgia.[1-2] Carbamazepine is related chemically to the tricyclic antidepressants. It is a derivative of iminostilbene with a carbamoyl group at the 5 position; this moiety is essential for potent antiseizure activity.[3-4] It is a white or almost white, crystalline powder, practically insoluble in water, freely soluble in methylene chloride, sparingly soluble in acetone and in alcohol, practically insoluble in ether. It shows polymorphism. Carbamazepine is official in IP, USP, BP etc.[5-6] As per investigation of literature, the UV spectro-photometric, HPLC analytical method were developed on different wavelength for analysis of Carbamazepine in plasma fluids, Human serum, Plasma and pharmaceutical tablet dosage form or bulk drug samples.[7-11] The rational of this work to develop a simple, accurate, rapid, precise, reproducible and cost effective spectro-photometric method for the direct quantitative determination of carbamazepine. In this method, we developed a method for determination carbamazepine in bulk drug sample and tablet dosage form and validation as per International Conference on Harmonization (ICH) Guideline.

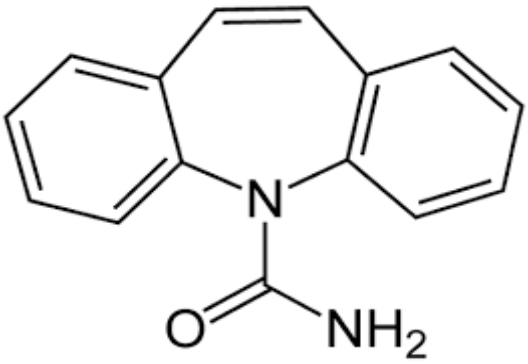

Fig. 1- Chemical structure of Carbamazepine.

MATERIALS AND METHOD

Materials

Carbamazepine was gifted from Swapnroop Drugs and Pharmaceutical, Aurangabad, India. The commercially available tablets Tegretol®CR 400 mg (Batch No.156014), Tegretol®CR 200 mg (Batch No.156021ME) Tegretol®. Methanol (HPLC Grade) was used as a solvent was obtained from Fisher Scientific, India and distilled water was used obtained from Water purification unit.

Instruments Used

Shimadzu UV1700 pharma spec double beam spectrophotometer with UV Probe software version 2 was used to develop the analytical method. The above instruments had automatic wavelength accuracy 0.1 nm and matched quartz cells with 1 cm cell path length, Ultrasonicator (Spectra lab UCB 40, India) and Weighing balance (Shimadzu, Japan) were used for this work.

METHOD DEVELOPMENT

Preparation of standard stock solution

A Standard stock solution was prepared by accurately weighed 25 mg of carbamazepine in 25 ml of volumetric flask and dissolved in Methanol to obtain a concentration 1 mg/ml or 1000 µg/ml (standard Stock I). Further diluting 2.5 mL of stock solution to 25 ml mixture of Methanol: water (50:50 v/v) to get desired concentration of 100 µg/ml (standard Stock II).

Selection of wavelength for analysis of carbamazepine

Accurately measured 1 ml of standard stock II solution was transferred into 10 ml volumetric flask and diluted to 10 ml to give concentration of 10 µg/ml and it was used for initial spectral scan in the UV range of 400-200 nm to detect maximum wavelength and further dilutions for linearity were prepared from the stock solution by allegation method.

Preparation of serial dilutions

The serial dilutions were prepared from the standard stock II solution to get a respective concentration of 2, 4, 6, 8, 10 &12 µg/ml.

Method Validation

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, ruggedness, sensitivity and specificity according to ICH Q2 (R1) guideline and USP guidelines.[12-13]

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which are directly proportional to the concentration of an analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of an analyte in the sample for which it has been demonstrated that the analytical procedure has

a suitable level of precision, accuracy and linearity. The linearity of the analytical method was demonstrated over the concentration range investigated by triplicate analysis (n = 3) at a concentration range of 2-12 µg/ml. The absorbance obtained at respective concentration was recorded, and the graph is plotted as concentration (µg/ml) versus absorbance. The linear regression equation and the coefficient correlation were obtained from the UV probe software.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. The accuracy of proposed method was determined on the basis of recovery study. Recovery study was carried out by spiking standard working solution to sample solution (formulation) at three different levels 80%, 100% and 120%. The final concentration of carbamazepine was determined at each levels of the amount; three determinations were performed. The percentage recovery was calculated as mean±standard deviation.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day precision study, three different solutions of same concentration were prepared and analysed in the same day (morning, noon and evening), whereas in the inter-day precision study, the solutions of same concentration were prepared and analysed, for three consecutive days, and the absorbances were recorded. All study was performed in triplicates. The result was indicated by calculating % RSD.

Ruggedness

The ruggedness is a degree of reproducibility of test result under verification of condition like a different analyst, different instruments and different days. To establish ruggedness of the proposed method, the solutions of 4 µg/ml of standard carbamazepine solution was prepared and analysed with the change in the different analyst.

Limit of Detection (LOD)

The LOD of the developed UV method was calculated by using following formula

$$LOD=3.3\times SD/S$$

Where, SD= Standard deviation of Y-intercepts

S= Slope

Limit of Quantitation (LOQ)

The LOQ of the developed UV method was calculated by using following formula

$$LOQ= 10\times SD/S$$

Where, SD= Standard deviation of Y-intercepts

S= Slope

Assay of marketed tablet formulation

The Carbamazepine content in its marketed formulation (Tegretol®CR 400 mg & Tegretol® CR 200 mg) was estimated using pre-validated UV spectrophotometric method. Twenty tablets were accurately weighed, and average weight was calculated, they were crushed to fine powder. The powder equivalent to 25 mg carbamazepine was dissolved in 15 ml of methanol with the help of sonication and volume was made up using methanol up to the mark of 25 ml volumetric flask. The solution was filtered using Whitman filter paper. This solution was further diluted to obtain 10 µg/ml concentration of the solution by using distilled water as a solvent (n=5) and observed by UV analysis.

RESULTS AND DISCUSSION

Selection of wavelength

The spectra of carbamazepine in methanol showed absorption at 284 nm shown in fig. 2, which is complying with reported λmax. Hence, it was selected as λmax of carbamazepine in methanol: distilled water (50:50 v/v) for further use.

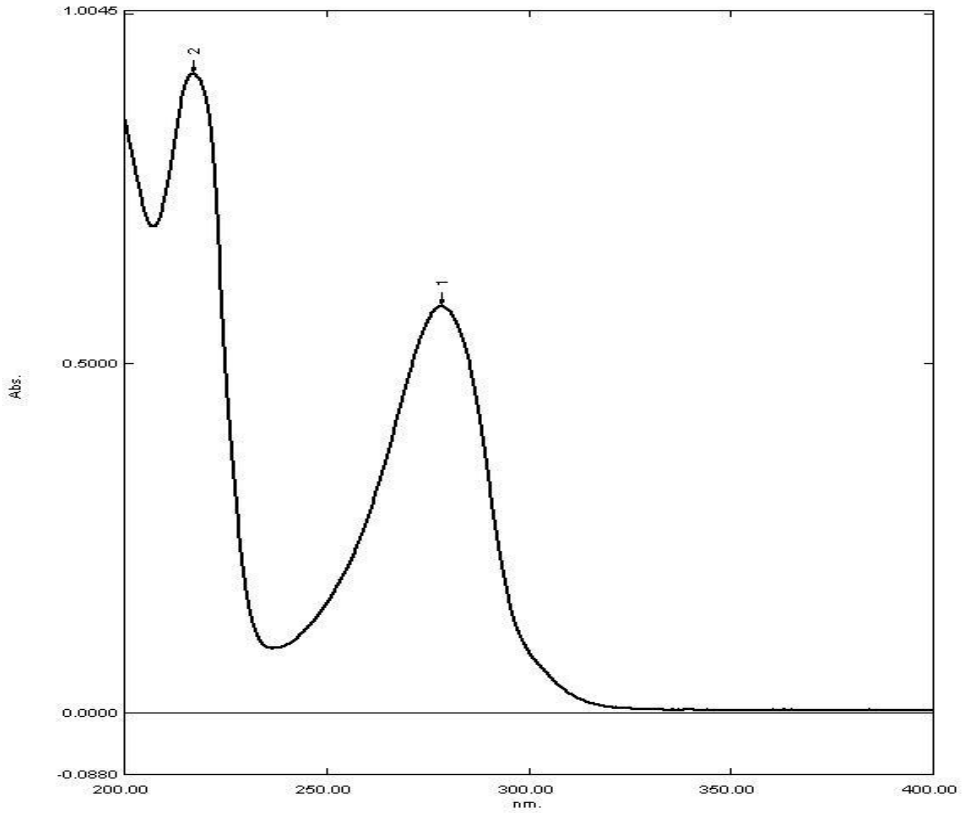

Fig. 2: UV spectrum of Carbamazepine.

Preparation of calibration curve

Quantification of unknown samples by UV-Visible spectrophotometer or any other instrumental method of analysis needs a reproducible calibration curve and an equation stating correlation between concentration and the response. As compare to graphical method, above stated method is widely accepted and reproducible in nature. Considering the utility of quantitative analysis of carbamazepine, calibration curve for Carbamazepine was developed using seven different calibration standards. The absorbance of different calibration standards at 284 nm was recorded using fixed wavelength mode of UV-Visible spectrophotometer. Calibration curve was repeated five times and the mean values ± deviation was reported as shown in Table 1.

Table 1: Calibration standard data for Carbamazepine.

Concentration (µg/ml)	Absorbance
2	0.221±0.0029
4	0.395±0.0041
6	0.566±0.0052
8	0.725±0.0059
10	0.886±0.0066
12	1.033±0.0065

METHOD VALIDATION

Linearity and Range

Linearity and range are the key parameters of analytical method that demonstrates the limit within which the intended method is to be used for its optimum performance. Considering the prime importance of linearity and the range, seven point calibration curve of carbamazepine covering a range of 2-12 µg/ml was plotted. Details of concentrations and the respective mean absorbance values are depicted in Table 1. Calibration curve when subjected to least square regression analysis yielded an equation; y = 0.0813x+0.0685 with correlation coefficient 0.999 shown in Figure 3. From the linearity study, it was revealed that, developed UV method was linear in the pre-defined concentration range of calibration standards.

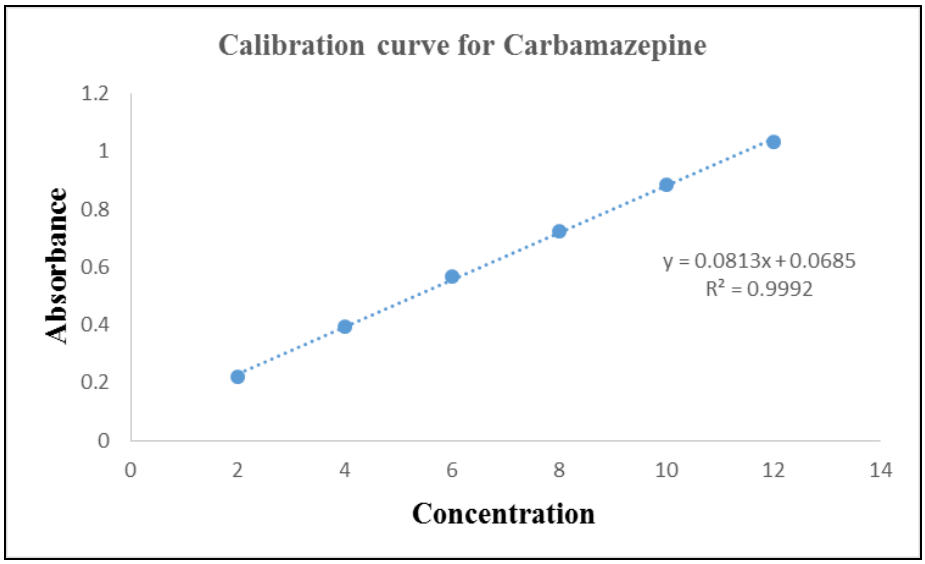

Fig. 3: Calibration curve for Carbamazepine.

Accuracy

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, results obtained would be reliable. In case of UV method for Carbamazepine, accuracy was established using recovery studies. At 80 % standard addition, mean recovery of Carbamazepine was found to be 99.60% whereas at 100 and 120 % standard addition, it was found to be 99.06 and 99.30% respectively. % RSD was found to be less than 2 for the Carbamazepine recovery studies as shown in Table 2. From the results of accuracy studies, it was observed that developed UV method is highly accurate as the percent recovery was in between 98 to 102% and the % RSD was well below 2%.

Table No 2: Accuracy data of UV method for Carbamazepine.

S No.	Concentration (%)	Origin level (µg/ml)	Amount added (µg/ml)	% Recovery	Mean % Recovery	% RSD
1	80	4	3.2	99.2188	99.60	0.3922
2	80	4	3.2	99.6094		
3	80	4	3.2	100.0000		
4	100	4	12	99.0625	99.06	0.9464
5	100	4	12	100.0000		
6	100	4	12	98.1250		
7	120	4.8	28.8	98.4375	99.30	0.8011
8	120	4.8	28.8	99.4792		
9	120	4.8	28.8	100.0000		

Precision

Precision is a measure of degree of scatter. It expresses the reproducibility of the measurements. It is expected that an analytical method should generate outcomes that are reproducible. Precise analytical method leads to accurate results. Considering the importance of reproducible yet accurate results, intra- and inter-day precision of developed UV method was established at 4, 8 and 10 µg/ml levels of Carbamazepine. The results in terms of mean absorbance values, percent assay and % RSD for the intra- and inter-day precision study are demonstrated in Table 3 and Table 4 respectively. % RSD values of intra-day precision study were found to be in between 0.56 and 0.82 whereas those of inter-day precision study were in between 0.36 and 0.59. Overall, % RSD values of less than 2 showed the precision of developed UV method.

Table No 3: Intra-day precision data of UV method for Carbamazepine.

	Morning			Afternoon			Evening		
Concentration Range (µg/ml)	Amt. found	% Assay	% RSD	Amt. found	% Assay	% RSD	Amt. found	% Assay	% RSD
4	3.93	98.25	0.5669	3.96	99.12	0.66	3.93	98.25	0.8294
8	7.93	99.12	0.6812	8.03	100.43	1.02	7.97	99.70	0.9048
10	9.93	99.30	0.9854	9.96	99.65	0.80	9.96	99.65	1.25

Table 4: Inter-day precision data of UV method for Carbamazepine.

	Day 1			Day 2			Day 3		
Concentration Range (µg/ml)	Amt. found	% Assay	% RSD	Amt. found	% Assay	% RSD	Amt. found	% Assay	% RSD
4	4	100	0.3691	3.9651	99.12	0.5737	3.93	98.25	0.5923
8	7.98	99.85	0.6589	7.9302	99.12	0.9457	7.95	99.41	0.4579
10	9.93	99.30	0.8945	10.0116	100.11	1.05	9.87	98.72	0.8648

Ruggedness

Ruggedness of analytical method is the degree of reproducibility of test results obtained by analysis of the same samples under a variety of conditions, such as different laboratories, different analyst, different instruments, different lots of reagent, different temperatures etc.. In order to determine the ruggedness of proposed spectrofluorimetric method, carbamazepine solutions were prepared and analyzed by different analysts. Sample analysis and data processing resulted into % RSD values between 0.30 and 0.34. Results of ruggedness studies revealed that proposed spectrofluorimetric method was rugged as it showed % RSD values less than 2 (Table 5).

Table 5: Ruggedness data of Spectrofluorimetric method for Carbamazepine.

S. No.	Concentration (µg/ml)	Analyst	Amt. found	% Amt. found	% RSD
1	4	I	3.99	99.80	0.3008
2	4	II	3.98	99.60	0.3409

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

LOQ represents the lower most concentration that can be analyzed with acceptable accuracy and precision. Generally, LOQ is the first calibration standard. LOD and LOQ of proposed UV method was found to be 0.456 and 0.956 µg/ml respectively as shown in Table 6. Lower LOQ value indicated that proposed method would be suitable for analyzing the samples containing even small quantities of Carbamazepine.

Table No. 6: LOD, LOQ data of Carbamazepine.

1	LOD	0.456 µg/ml
2	LOQ	0.956 µg/ml

Estimation of Carbamazepine content in marketed formulation

The developed UV method was successfully applied for estimation of carbamazepine content in Tegretol®CR 400 mg and Tegretol®CR 200 mg. By proposed UV method, carbamazepine content in the tablet was found to be 98.28± %. & 99.84±% respectively.

CONCLUSION

The simple, rapid, precise, and economical spectrophotometric method has been developed for the quantitative estimation of carbamazepine in Bulk and pharmaceutical formulation. The method is validated as per the ICH and USP guidelines, and it is found that the developed method is robust and sensitive. Hence, this method can be successfully and

suitably acquired for routine quality control analysis of carbamazepine in bulk and pharmaceutical dosage form.

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