International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 2, 2016

Original Article

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

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Received: 03 Nov 2015 Revised and Accepted: 19 Dec 2015

ABSTRACT

Objective: To develop and validate simple, accurate, rapid, precise, reproducible and cost effective spectrophotometric method for the quantitative estimation of carbamazepine in a pharmaceutical formulation.

Methods: The developed UV spectrophotometric method for the quantitative estimation of carbamazepine is based on measurement of absorption at maximum wavelength 284 nm using methanol as a solvent. The stock solution of carbamazepine was prepared, and subsequent suitable dilution was prepared in distilled water to obtained standard curve. The standard solution of carbamazepine shows absorption maxima at 284 nm.

Results: The drug obeyed beer lambert's law in the concentration range of 2-14 µg/ml with regression 0.9997 at 284 nm. The overall % recovery was found to be 99.99% 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 0.1568 and 0.1746 respectively which is<2% hence proved that method is precise.

Conclusion: 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: Carbamazepine, UV Spectrophotometry, Method development, Validation, ICH guidelines, Methanol.

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. [5-7]. 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 [8-11]. 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 [12].

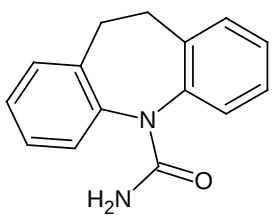

Fig. 1: Chemical structure of carbamazepine

MATERIALS AND METHODS

Instrument

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.

Material

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® Chewable 100 mg (Batch No.142009EH) Novartis India Ltd. were obtained from the market. Methanol (HPLC Grade) was used as a solvent was obtained from Fisher Scientific, India. The Aerosil 200 was obtained from Evonik Industries and Distilled water was used obtained from Water purification unit.

Method development

Preparation of Standard 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 with distilled water to get desired concentration of 100 µg/ml (standard Stock II) [12-14].

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 up to 14 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 [15-16].

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 2-14 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.

Limit of detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantitated as an exact value.

The limit of detection (LOD) was determined by preparing solutions of different concentrations from 2-14µg/ml.

LOD = 3.3 σ/S

Where,

σ=Standard deviation

S= Slope

Limit of quantification (LOQ)

The detection limit is the lowest amount of analyte in a sample which can be detected but not quantitates. The LOQ was calculated using the formula involving the standard deviation of response and the slope of the calibration curve.

LOD = 10 σ/S

Where,

σ=Standard deviation

S= Slope

Sensitivity

The sensitivity of the method was determined by calculating the different parameter like molar absorptivity and Sandell's sensitivity.

Robustness

The robustness of an analytical procedure is a measure of its capacity remains unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the proposed method the solutions of 2µg/ml of standard carbamazepine solution was prepared and analysed by a change in wavelength. The wavelength was selected λmax ± 1 i.e. 283 and 285 nm respectively for standard carbamazepine solution.

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 2 µg/ml of standard carbamazepine solution was prepared and analysed with the change in the different analyst.

Specificity

Specificity is the ability to assess the analyte unequivocally in the presence of components which may be expected to be present. Typically these might include impurities, degradant, matrix, etc. For this study specificity was done by using an excipient Aerosil 200. The three different concentrations at three levels 80%, 100%, 120% respectively of standard carbamazepine (5µg/ml) spiked on Aerosil sample. At each level of the amount, the triplicate study was performed to check the effect of Aerosil 200.

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 for further use.

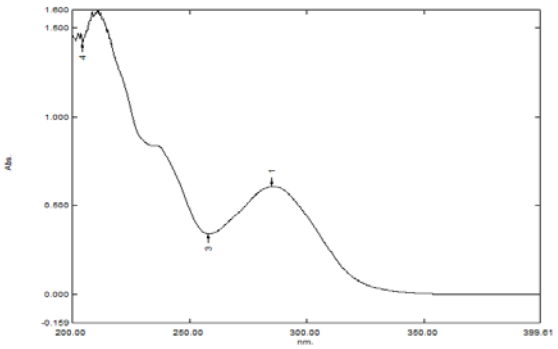

Fig. 2: UV spectrum of carbamazepine

Linearity and range

The linearity for the developed method was investigated by replicate analysis (n=3) at seven concentration levels (2-14 µg/ml) of

reference standard carbamazepine. The absorbance obtained at respective concentration was recorded and graph was plotted shows good linear correlation coefficient from the UV probe software. The linearity was shown in table 1 and fig. 3.

Table 1: Calibration curve data of carbamazepine

Concentration (µg/ml)	Absorbance
0	0
2	0.13
4	0.23
6	0.35
8	0.47
10	0.59
12	0.70
14	0.82

Accuracy

The accuracy was determined in triplicate by analysing % recovery of carbamazepine by standard addition method. The percent recovery obtained indicates non-interference from the excipients used in the formulation. The results were shown in table 2.

Method precision

The precision of proposed method was determined by Intra-day and Inter-day precision, and it was expressed in terms of percent relative standard deviation (%RSD). For Inter-day and Intra-day %RSD were found in the range of 0.1568 and 0.1746 respectively as shown in table 3.

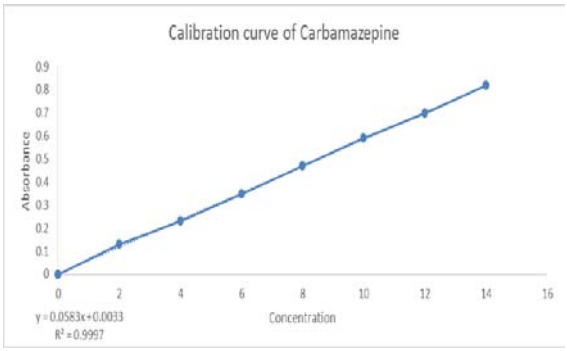

Fig. 3: Calibration curve of carbamazepine

Table 2: Accuracy results of carbamazepine

Level of addition (%)	Standard API (µg/ml)	Formulation stock (µg/ml)	Total concentration (µg/ml)	Drug recovered (µg/ml)	%Recovery	Mean % recovery ±SD	%RSD
	5	4	9	8.987	99.8555		0.0513
80	5	4	9	8.987	99.9444	99.9148±0.0513	
	5	4	9	8.995	99.9444		
	5	5	10	10.022	100.22		0.0641
100	5	5	10	10.01	100.1	100.1733±0.0642	
	5	5	10	10.02	100.2		
	5	6	11	10.926	99.3272		0.8855
120	5	6	11	11.102	100.9273	99.9090±0.8847	
	5	6	11	10.942	99.4727		

Table 2: Inter-day precision

Mean absorbance	mean±SD	%RSD	Mean %RSD
0.8256	0.8244±0.0012	0.1526	
0.8231			
0.8246			
0.8256	0.8245±0.0014	0.1742	
0.8251			0.1568
0.8229			
0.8245	0.8238±0.0011	0.1437	
0.8225			
0.8246			

Table 3: Intra-day precision

S. No.	Mean Absorbance	mean±SD	%RSD	Mean %RSD
	0.8255	0.8244±0.0016	0.2058	
1	0.8231			
	0.8246			
	0.8251	0.8245±0.0014	0.1742	
2	0.8256			0.1746
	0.8229			
	0.8245	0.8238±0.0011	0.1437	
3	0.8225			
	0.8246			

Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of detection

The limits of detection (LOD) which represent the sensitivity of the proposed method were determined. The LOD value obtained was 0.4439 µg/ml. It indicates the high sensitivity of the proposed method.

Limit of quantitation

The limits of Quantitation (LOQ) which represent the sensitivity of the proposed method were determined.

The LOQ value obtained was 1.267 µg/ml. It indicates the high sensitivity of the proposed method.

Ruggedness

In the ruggedness study, the influence of small, deliberate variations of the analytical parameters on the absorbance of the drug was examined. The factor selected was a change in the analyst. The result of ruggedness study indicates that the selected factor remained unaffected by small variations with % RSD of 0.4429-0.4452, which confirms the ruggedness of method. The results were summarized in table 5.

Table 4: Ruggedness data for carbamazepine

Observation	Analyst 1	Analyst 2
Absorbance	0.13	0.13
Mean*	0.1303	0.1296
SD	0.0005	0.0005
%RSD	0.4429	0.4452

*(n=3)

Table 5: Robustness data for carbamazepine

Wavelength (nm)	Absorbance	Mean absorbance±SD	%RSD
283	0.1302		
283	0.1300	0.1301±0.0001	0.0768
283	0.1301		
284	0.1301		
284	0.1303	0.1301±0.00011	0.0887
284	0.1301		
285	0.1301		
285	0.1300	0.1301±0.0001	0.0768
285	0.1302		

Robustness

Robustness of this method was determined by analysing the standard carbamazepine solution of 2 µg/ml at a different wavelength (i.e. λ max±1). Absorbance was measured. The standard deviation and percent relative standard deviation was calculated. Results of robustness study indicate that the selected factor remained unaffected by small variation with RSD 0.0768-0.0887 confirms the robustness of the method. Results were shown in table 6.

Sensitivity

The developed method showed the high molar absorptivity value i.e. 15471, which indicated that it absorb light very effectively at the appropriate wavelength and hence low concentrations of a compound can be easily detected. Sandell's sensitivity value i.e. 0.0152 µg/cm^2 suggested that the carbamazepine can be detected in the very low concentration at a path length of 1 cm. Both the above parameter will prove the sensitivity of drug and method developed at the specific wavelength at a specific concentration.

Specificity

The specificity of proposed method was ascertained by performing the study at three concentration levels of aerosol 200 i.e. 80%, 100% and 120%. The percent recovery obtained indicates non-interference from the excipients in the formulation. The results of specificity study were given in table 7.

Table 6: Specificity study

Level of addition	Standard API (µg/ml)	Aerosil 200 (µg/ml)	Total concentration (µg/ml)	Absorbance	Drug recovered (µg/ml)	% Recovery	Mean % Recovery
	5	4	9	0.2958	5.00	100.00	
80	5	4	9	0.2958	5.00	100.00	100.00
	5	4	9	0.2959	5.001	100.0338	
	5	5	10	0.2958	5.00	100.00	
100	5	5	10	0.296	5.0033	100.0676	99.9887
	5	5	10	0.2955	4.9949	99.89858	
	5	6	11	0.2959	5.0016	100.0338	
120	5	6	11	0.2956	4.9966	99.93238	100.00
	5	6	11	0.2959	5.0016	100.0338	

Table 7: Assay of tablet dosage form

Brand name	Sample solution concentration (µg/ml)	Amount found (%)	Mean±SD (%)	%RSD
Tegretol®CR400 mg Novartis India Ltd.	10	100.23	100.15±0.1473	0.1470
	10	100.24		
	10	99.98		
Tegretol®CR200 mg, Novartis India Ltd.	10	100.1	100.08± 0.1205	0.1204
	10	99.96		
	10	100.2		
Tegretol®100 mg Chewable, Novartis India Ltd.	10	100.59	100.22± 0.3507	0.3499
	10	100.20		
	10	99.89		

Table 8: Summary of validation parameters

Parameters	Results
λmax (nm)	284
Linearity range (µg/ml)	2-14
Regression equation	y = 0.0583x+0.0033
Correlation coefficient (r^2)	0.9997
Precision	
Inter-day precision (%RSD)	0.1568
Intra-day precision (%RSD)	0.1746
LOD (µg/ml)	0.4439
LOQ (µg/ml)	1.267
Ruggedness(%RSD)	0.4429-0.4452
Robustness (% RSD)	0.0768-0.0887
Molar absorptivity	15471 L/mol
Sandell's sensitivity	0.0152µg/cm^2
Accuracy (%)	99.99
Specificity(%RSD)	0.0195-0.0850
Assay of marketed formulation (%)	100.15

Assay of marketed tablet formulation

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 Whatman filter paper. This solution was further diluted to obtain 10 µg/ml concentration of the solution by using distilled water as a solvent and observed by UV analysis. This procedure was repeated in triplicate.

The observed assay for commercially available tablets Tegretol®CR 400 mg, Tegretol®CR 200 mg and Tegretol® Chewable 100 mg and validation parameters were summarized in table 8 and 9 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.

CONFLICT OF INTERESTS

Declared none

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