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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF CARBAMAZEPINE IN BULK AND PHARMACEUTICAL PREPARATION

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ABSTRACT

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for determination of Carbamazepine in tablet dosage form. The separation was carried out using a mobile phase containing methanol and Water with pH 3.5 adjusted by 20% of Phosphoric acid in the ratio of 80:20 v/v. Various types of analytical columns were tested for separation of carbamazepine. The good separation of chromatographic peaks was observed by using Intersil ODS C18 (150 × 4.6-mm, 5µ) column with flow rate of 1 mL / min using UV detection at 285 nm. The described method was linear over a concentration range of 3.12-25 µg/mL (r^2>0.9992). Separation was achieved within 5 min. The mean % recovery was found to be 100.27 % .The limit of detection (LOD) was found to be 0.25 µg/mL. Whereas the limit of quantification (LOQ) was found to be 0.77 µg/mL. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, accurate and cost effective which is useful for the routine determination of Carbamazepine in bulk drug and in its tablet dosage form.

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INTRODUCTION

Carbamazepine (CBZ) 5*H*-Dibenz [*b*,*f*]azepine–5–carboxamide (Figure:1),an anticonvulsant, is effective in the treatment of grandmal and psychomotor epilepsy [1]. In addition, it is the drug of choice in trigeminal neuralgia. The important metabolic pathway yields carbamazepine-10, 11-epoxide, an active metabolite shown to be potent as parent drug in animal models of epilepsy [2-3]. A CBZ plasma concentration ranging from 4 to 12 µg/mL is associated with seizure control. CBZ is a strong inducer of microsomal enzymes (cytochrome P450 in liver) which can quicken its own metabolism and those of co-administered drugs. Hence, polytherapy may be associated with drug interactions and undesired toxicity [4-8]. Carbamazepine having low water solubility but has lipophilicity. The drug is available for use in the

tablet and suspension form in various dose. Liquid chromatography is an official method for determination of carbamazepine [9]. The various methods were investigated by researchers for quantitative estimation of carbamazepine such as Thin Layer Chromatography, Gas Chromatography, Planar Chromatography, High Performance Thin Layer Chromatography, FT-Raman Spectroscopy [10-16]. Unfortunately All these methods which are used for analysis are expensive, highly sophisticated, time consuming. The spectrometric methods were used early for the estimation of an drug but due to the lack of accuracy and repeatability these methods does not used for routinely estimation of drug. In view of this, Reverse Phase High Performance chromatography (RP-HPLC) considered as an important alternate tool for analysis of drug from pharmaceutical preparations. Due to the high sensitivity, accuracy and ruggedness RP-HPLC was considered as an useful method for estimation of carbamazepine[17]. Therefore the purpose of this research to develop and validate RP-HPLC method for estimation of carbamazepine from pharmaceutical preparations. The method was subjected to validation according to US Food and Drug Administration (FDA) and International Conference on Harmonisation (ICH) guidelines [18, 19]. It fulfils the validation criteria in every segment of validation. Finally, the developed method was successfully applied to routine analysis of Carbamazepine. It could be used for cost-effective therapeutic drug monitoring of carbamazepine due to its appropriate sensitivity and selectivity.

MATERIALS AND METHODS

Reagents and Materials

Methanol (HPLC grade) was procured from Thomas baker Chemicals, Mumbai. Orthophosphoric acid (AR grade) was purchased Poona Chemicals, Pune. Carbamazepine was obtained as a gift sample from Sun Pharmaceuticals, Mumbai. The commercial preparation of Carbamazepine is available in the tablet dosage (Tegrital® 100 Novartis India Ltd) form in the market.

INSTRUMENTATION

JASCO HPLC 2000 series was used for analysis. The method was carried out on Intersil ODS C18 (150 × 4.6-mm, 5µ) column as stationary phase. Borwin software provided by JASCO 2000 series was used throughout this experiment. The injection volume was 20 µL.

Optimized chromatographic conditions

The mobile phase comprised of methanol and Water with pH 3.5 adjusted by 20% of Phosphoric acid in the ratio of 80:20 v/v. The mobile phase was filtered through 0.45 µm membrane filter and was degassed before use.

Preparation of Carbamazepine standard stock solution

A stock solution of Carbamazepine (1000 µg/mL) was prepared by taking 10 mg of drug, accurately weighed in separate 10 mL volumetric flask. They were dissolved in 2.5 mL of mobile phase and then the volume was made by mobile phase up to the mark. Drug, appropriate aliquots was pipette out from the standard stock solution into a series of 10 mL volumetric flasks to get a concentration of 3.12, 6.25, 12.5, 18.75 and 25 µg/mL of Carbamazepine

Preparation of sample solution

Twenty tablets (Tegrital®) were weighed and powdered. The quantity of the powder equivalent to 10 mg of Carbamazepine was transferred to a 100 mL volumetric flask. and dispersed in 50 mL methanol, sonicated for 20 min to dissolve the drug as completely as possible and the volume was adjusted up to the mark with methanol (100 µg/mL). The solution was then filtering through Whatman filter paper no. 41.

Determination of analytical wavelength

The standard solution of Carbamazepine was injected under the chromatographic condition described above. The elution showed reasonable good response at 285 nm using UV detector. So drugs were detected at the common analytical wavelength.

Validation of Proposed HPLC method

System suitability

System suitability tests were performed including resolution, tailing factor, number of theoretical plates, capacity factor etc.

Linearity

The developed method has been validated as per ICH guidelines. Every 20 µL of the solution of Carbamazepine in concentration range of 3.12 to 25 µg/mL were injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curve of Carbamazepine was obtained by plotting the peak area ratio versus the applied concentrations of Carbamazepine.

Precision

Repeatability of the method was checked by injecting triplicate injections of the solution 12.5 µg/mL of Carbamazepine and the % RSD was found to be 0.6 %. Variability of the method was studied by analyzing the solution on the same day (intra-day precision) and on three different days (inter-day precision). The results obtained for intra-day precision (% RSD) was 0.7%, at n = 6, for Carbamazepine. The inter-day precisions (% RSD) was 0.7 %, at n = 6, for Carbamazepine.

Recovery studies

Recovery experiments were carried out to check for the presence of positive or negative interferences from excipients present in the formulation and to study the accuracy and precision of the method. Recovery experiment was performed by the standard addition method. The recovery of the added standard was studied at three different levels viz 80%, 100% and 120% of the estimated amount of the drug. Each set of recovery of added standard was calculated.

The Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated as 3.3 ∂/S and 10 ∂/S respectively as per ICH guidelines, where ∂ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Robustness

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated by calculating % RSD.

Analysis of Carbamazepine in Dosage form

The response of sample solution was measured at 285 nm under the chromatographic condition mentioned above for quantitation of Carbamazepine. The amounts of the Carbamazepine present in the sample solution was determined by fitting the responses into the regression equation for Carbamazepine.

RESULTS AND DISCUSSION

Method Development and optimization

The method developed was carried out on Intersil ODS C18 (150 × 4.6-mm, 5µ) column as stationary phase and various mobile phases were prepared by mixing solvents methanol and water (80:20) v/v ratio. The pH adjusted to (3.5 ±0.2) with O-Phosphoric acid. The prepared mobile phase was filtered through a Millipore 0.45 µm membrane filter and ultrasonically degassed prior to use. The detection wavelength was set at 285 nm. The elution was done at a flow rate of 1.0 mL/min under ambient condition.

Under these chromatographic conditions, Carbamazepine peaks were well resolved and their retention times were found to be 2.6 min. A typical chromatogram of the drugs is illustrated in Figure: 2. The % assay or average amount of Carbamazepine found to be 100.89% (Table 1). System suitability tests including resolution, tailing factor, number of theoretical plates, capacity factor were carried out and results are shown in Table 2. All of these results were acceptable in their limits defined by official guidelines [19].

Validation of Proposed method

Linearity

Calibration graph was found to be linear at range 3.13 to 25 µg/mL for the Carbamazepine. Regression analysis of the calibration data Carbamazepine showed that the dependent variable (peak area) and the independent variable (concentration) were represented by the equations. For Carbamazepine the correlation of coefficient (r^2) obtained was found to be 0.9992 in Figure: 3 (Table 3).

Precision

The repeatability results were found to be 0.6 % for Carbamazepine. The results obtained inter-day precisions (% RSD) was 0.7 % for Carbamazepine. The results obtained for intra-day precision (% RSD) was 0.7% Carbamazepine.

Recovery

The mean % recovery was found to be 100.27 % for Carbamazepine. The amounts recovered Carbamazepine and the values of percent recovery were calculated, results are shown in Table 4

LOD and LOQ

The limit of detection (LOD) for Carbamazepine was found to be 0.25 µg/mL. Whereas the limit of quantification (LOQ) was found 0.77 µg/mL.

Robustness

The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2 %.

CONCLUSION

The present work was undertaken with an aim to meet these challenges of analytical chemist. RP-HPLC method was developed for Carbamazepine and was validated as per ICH guidelines. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, accurate and cost effective which is useful for the routine determination of said drug in bulk as well as its tablet dosage form.

FIGURES

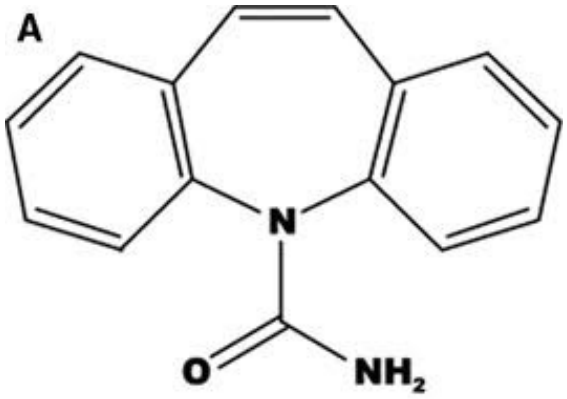

Figure: 1 Chemical structure of Carbamazepine

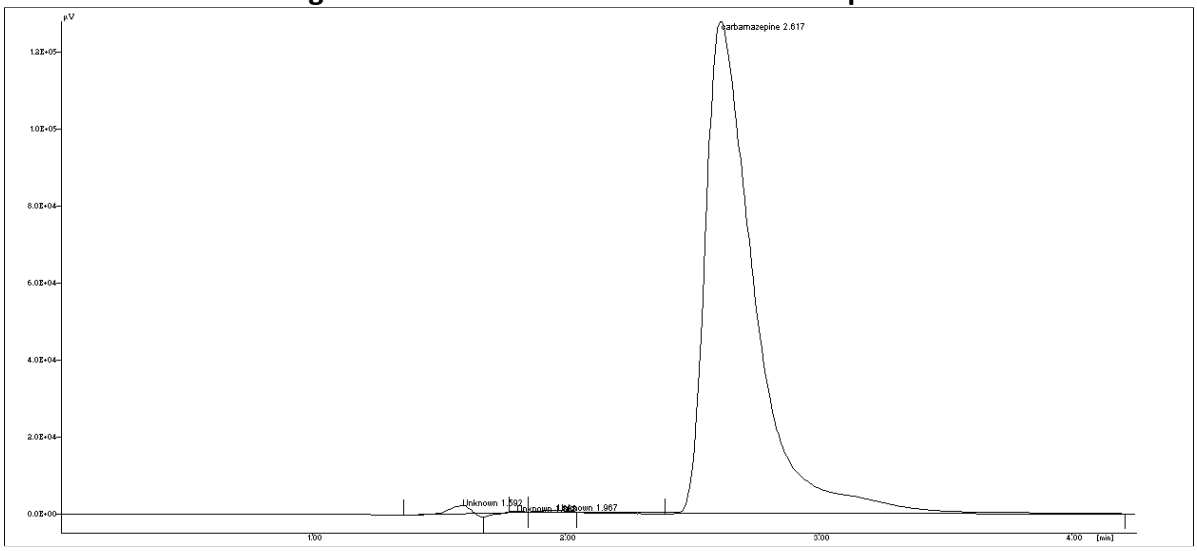

Figure: 2 Representative HPLC chromatogram of Carbamazepine.

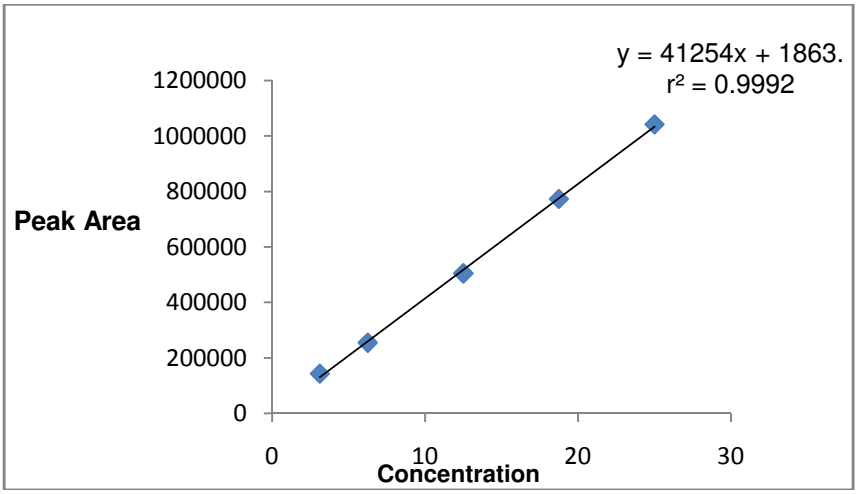

Figure: 3: Linearity graph for Carbamazepine.

TABLES

Table 1: Assay of Carbamazepine

Drug	Labeled claim (mg)	Amount found(mg)	%RSD	% Assay
Carbamazepine	12.50	12.61	1.5	100.89

Table 2: System suitability test for Carbamazepine

Parameter	Carbamazepine
Resolution (R)	4.79
Asymmetry (A_s)	1.81
No. of theoretical plates (N)	3457
Capacity factor (K')	3.8

Table 3: Regression Analysis Data and Summary of Validation Parameters

Parameters	Carbamazepine
Detection Wavelength (nm)	285
Linearity range (µg/mL)	3.12-25
Slope	41254
Intercept	1863
r^2	0.9992
LOD (µg /mL)	0.25
LOQ (µg /mL)	0.77
Intra day (% RSD)*	0.7
Inter day (% RSD)*	0.7
Repeatability (% RSD)*	0.6
Mean Recovery	100.27%

Table 4: Recovery studies for Carbamazepine

Conc. of Std. Solution (µg/mL)	Conc. of sample solution (µg/mL)	Amount found (µg/mL)	% Recovery	% RSD
6.25	5	11.17	99.31	1.3
6.25	6.25	12.66	101.32	1.1
6.25	7.5	13.77	100.18	0.8

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