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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF CARBAMAZEPINE IN BULK DRUG AND FORMULATIONS

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Keywords:

Carbamazepine, HPTLC, Methanol, Bulk drug, Formulations

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ABSTRACT: A new, simple, and rapid high-performance thin-layer chromatographic method was developed and validated for quantitative determination of Carbamazepine. Carbamazepine was chromatographed on silica gel 60 F254 TLC plate using Ethyl acetate: Methanol (9: 1 v/v) as mobile phase. Carbamazepine was quantified by densitometric analysis at 239 nm. The method was found to give compact spots for the drug. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range 100 - 600 ng/spot. The method was validated for precision, recovery, accuracy, and robustness as per the International Conference on Harmonization guidelines. The minimum detectable amount was found to be 15.096 ng/spot, whereas the limit of quantitation was found to be 45.696 ng/spot. Statistical analysis of the data showed that the method is precise, accurate, reproducible, and selective for the analysis of Carbamazepine. The method was successfully employed for the estimation of equilibrium solubility, quantification of Carbamazepine as a bulk drug, in commercially available preparation.

INTRODUCTION: Carbamazepine (CBZ), 5-H-Dibenz [b.f] azepine-5-carboxomide, is widely prescribed as an for schizophrenia and schizoaffective psychoses and anticonvulsant, antiepileptic drug. Various methods have been reported for the determination of CBZ in pharmaceutical preparations including spectro-photometric methods, spectrofluorimetry method, gas-liquid chromatography (GC), FTIR, planar chromatography, and high performance liquid chromatography (HPLC). Most of the methods reported are highly sophisticated, costly, and time consuming and require special sample preparation.

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So simple method for the determination of carbamazepine using high performance thin layer chromatography (HPTLC) with ultraviolet absorbance detection (UV) was developed. The method was validated successfully for the determination of carbamazepine and found simple, rapid, and highly sensitive and it could be reliable for pharmacokinetic studies in humans [1].

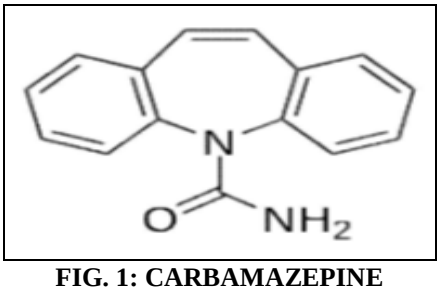

FIG. 1: CARBAMAZEPINE

Experimental: [2-6]

Apparatus: The HPTLC system (Hamilton, Bonaduz, Switzerland) consisted of CAMAG

Linomat5 sample applicator (Switzerland) connected to a nitrogen cylinder, a twin trough glass chamber (CAMAG, Muttenz, Switzerland) (10 × 10 cm), a derivatization chamber, and a plate heater. Pre coated silica gel aluminum plate 60 F254 TLC plates (10 × 10 cm, layer thickness 250 µm (E. Merck, Darmstadt, Germany) was used as stationary phase. TLC plates were prewashed twice with10mL of methanol and activated at 80 ºC for 5min prior to sample application. Densitometric analysis was carried out using a CAMAG TLC scanner at 239 nm with win CATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Reagents and Materials: Carbamazepine pure powder was obtained. Tablet formulation, Carbatol 200 (Torrent Pharmaceuticals Ltd., India) was obtained commercially were weighed and powdered. Methanol, ethyl acetate, distilled water was used throughout the study. All other chemicals and solvents were of analytical reagent grade.

HPTLC Method and Chromatographic Conditions:

Sample Application: The standard and formulation samples of CBZ were spotted on Precoated TLC plates in the form of narrow bands of lengths 6 mm, with 10 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas.

Mobile Phase and Migration: Plates were developed using mobile phase consisting of Ethyl acetate: Methanol (9: 1 v/v). Linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 20 min. The length of chromatogram run was 9 cm and development time was approximately 20 min.

After development, the TLC plates were dried completely.

Chromatogram and System Suitability Parameters of Drug: Under the optimized chromatographic conditions 200 ng/band of Carbamazepine was applied on TLC plate and the retention factor of repeated applications was found to be Carbamazepine = 0.78 ± 0.0052.

S. no.	R_f
1	0.78
2	0.77
3	0.78
4	0.77
5	0.78
7	0.78
AVG	0.78
ST. DEV	0.0052
% RSD	0.6649

Carbamazepine = 0.78 ± 0.0052

Densitometric Analysis and Quantitation Procedure: Densitometric analysis was carried out using a CAMAG TLC scanner at 239 nm with win CATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm. The spots were analyzed at a wavelength of 239 nm. The slit dimensions used in the analysis were length and width of 5 mm and 0.45 mm, respectively, with a scanning rate of 20 mm/s.

Preparation of CBZ Standard Stock Solution: Standard stock solution of Carbamazepine was prepared by dissolving 10 mg of drug in 10 ml of Methanol to get concentration of 1000 µg/ml. From the standard stock solution, 1 ml was further diluted to 10 ml with mobile phase to get 100 µg/ml solution of Carbamazepine.

Preparation of Sample Solution (Assay): Twenty tablets [Carbatol 200; Torrent Pharmaceuticals] were weighed and powdered. Tablet powder containing Carbamazepine equivalent to 10 mg of Carbamazepine was weighed and transferred to 10 ml volumetric flask and was diluted with Methanol. It was sonicated for 10 min. and filtered so as to get solution having concentration 1000 µg/ml. 1 ml of this solution was further diluted with methanol to get the final concentration 100 µg/ml of Carbamazepine. 2 µl volume of resulting solution was applied on TLC plate and developed under optimized chromatographic condition six determinations were carried out from homogenous sample to determine % assay **Table 1**.

Selection of Wavelength: From the standard stock solution further dilutions were done using mobile phase and scanned over the range of 200 - 400 nm and the spectrum was obtained. It was observed that Carbamazepine showed considerable absorbance at 284 nm **Fig. 2**.

Method Validation: Validation of the developed HPTLC method was carried out as per the ICH Q2 (R1) guidelines for specificity, accuracy, precision, repeatability, and robustness.

Specificity: The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.995, indicating the no interference of any other peak of degradation product, impurity or matrix.

Sensitivity: Sensitivity of the method was determined with respect to limit of detection (LOD) and limit of quantification (LOQ).

Linearity and Calibration Curve: From the standard stock solution (1000 µg/ml) of Carbamazepine further dilutions was made with methanol to get solution having concentration 100 µg/ml [100 ng/µl] Different volumes were applied on TLC plate to obtain linear range. Six replicates per concentration were applied. The linearity (relationship between peak area and concentration) was determined over the concentration range 100-600 ng/band. The results obtained are shown in **Table 2**. The peak area was plotted against the corresponding concentrations to obtain the calibration curve as shown in **Fig. 3**.

Accuracy: To check accuracy of the method, recovery studies were carried out by adding standard drug to sample at three different levels 50, 100 and 150%. Basic concentration of sample chosen was 200 ng/band of Carbamazepine from tablet solution. These solutions were applied under optimized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentrations of Carbamazepine were calculated by using linearity equation of Carbamazepine. The results obtained are shown in **Table 3**.

Precision: The precision of the method was demonstrated by Intra-day and Inter-day variation studies. In the Intraday studies, 3 replicates of 3 different concentrations (200, 400, 600 ng/band) of Carbamazepine were analysed in a day and percentage RSD was calculated. For the inter day variation studies, 3 replicates of different concentrations were analysed on 3 consecutive days and percentage RSD were calculated. The results obtained for Intraday and Inter day variations are shown in **Table 4** and **5**.

Robustness: Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase composition, chamber saturation period were altered and the effects on the area were noted. The results obtained are shown in **Table 6**.

RESULTS AND DISCUSSION:

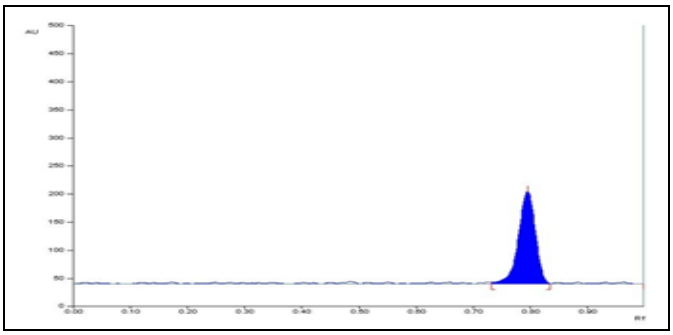

FIG. 1: CHROMATOGRAM OF STANDARD CARBAMAZEPINE (400 ng/band)

Selection of Wavelength: The spectra of carbamazepine in methanol showed absorption at 284 nm shown in **Fig. 2**, which is complying with reported λ_{max}. Hence, it was selected as λ_{max} of carbamazepine in methanol for further use.

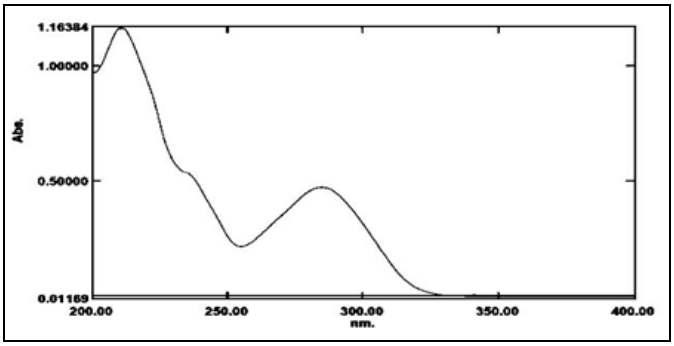

FIG. 2: UV SPECTRA OF CARBAMAZEPINE (10 µg/ml)

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

LOD is calculated from the formula:

$$LOD = 3.3\ \sigma / S$$

Where, σ = standard deviation of response for the lowest conc. in the range
S = slope of the calibration curve.

LOD of Carbamazepine = 15.079 ng/band

The quantitation limit is expressed as:

$$LOQ = 10\ \sigma / S$$

Where, σ = standard deviation of response for the lowest conc. in the range
S = slope of the calibration curve.

LOQ of Carbamazepine = 45.696 ng/band

Assay: The observed assay for commercially available tablets Carbatol 200 (Torrent Pharmaceuticals) was performed and % RSD were calculated which summarized in **Table 1**.

TABLE 1: ASSAY OF MARKETED FORMULATION

S. no.	Area	Concentration (ng/band)	% Recovery	% Recovery Mean ± SD	% RSD
1	5663	200.71	100.355	100.503	0.942
2	5638	199.324	99.662	± 0.947	
3	5703	202.927	101.464		
4	5640	199.435	99.717		
5	5718	203.759	101.879		
7	5648	199.878	99.939		

Linearity and Range: The linearity for the developed method was investigated by replicate analysis (n=7) at six concentration levels (100-600 ng/band). Method was found to be linearin a concentration range of 100–600 ng/band (*n* = 6), with respect to peak area. The linearity was shown in **Table 2** and **Fig. 3**.

Accuracy: The accuracy was determined in triplicate by analyzing % 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 3**.

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 0.3088 and 0.4287 respectively as shown in **Table 4** and **5**.

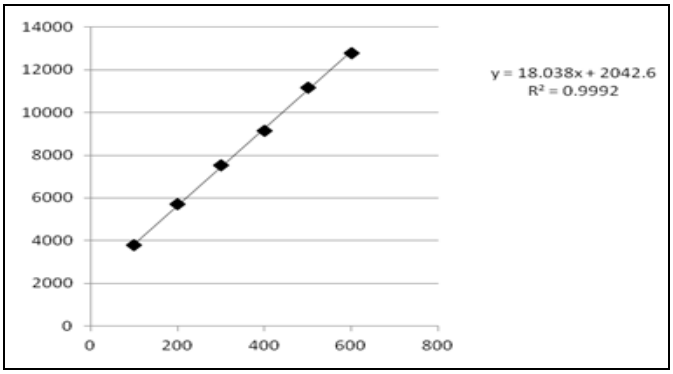

FIG. 3: CALIBRATION CURVE FOR CARBAMAZEPINE

TABLE 2: LINEARITY STUDY OF CARBAMAZEPINE

Replicates	Concentrations of Carbamazepine					
	100 ng/band	200 ng/band	300 ng/band	400 ng/band	500 ng/band	600 ng/band
	Peak Area					
1	3825	5719	7517	9145	11183	12828
2	3709	5708	7507	9131	11154	12809
3	3718	5735	7522	9134	11179	12837
4	3817	5757	7590	9104	11175	12772
5	3741	5707	7428	9122	11207	12815
7	3818	5727	7777	9242	11128	12737
Mean	3771.1	5708.7	7539.7	9146.3	11171	12799.3
Std. Dev.	52.97	52.48	84.37	48.83	27.08	38.11
%RSD	0.014	0.0091	0.0119	0.0053	0.00242	0.00298

TABLE 3: RECOVERY STUDY OF CARBAMAZEPINE

Level	Conc. (ng/band)		Area	Mean	Amount recovered (ng/band)	% Recovery
	Sample	Std				
50%	200	100	7549	7493	298.17	100.72
			7418			
			7512			
100%	200	200	9187	9214	402.97	99.40
			9247			
			9210			
150%	200	300	11075	11122	498.80	100.67
			11184			
			11109			

TABLE 4: INTRA-DAY PRECISION STUDY CARBAMAZEPINE

Concentration (ng/band)	Area (µV. Sec)	% Recovery ± SD	Mean % Recovery* ± SD	% RSD*
200	5719		100.37 ± 0.31	0.3088
200	5708	101.95 ± 0.37		
200	5735			
400	9245			
400	9204	99.62 ± 0.31		
400	9242			
600	12828			
600	12772	99.44 ± 0.27		
600	12815			

TABLE 5: INTER-DAY PRECISION STUDY CARBAMAZEPINE

Concentration (ng/band)	Area (µV. Sec)	% Recovery ± SD	Mean % Recovery* ± SD	% RSD*
200	5708		100.29 ± 0.47	0.4287
200	5726	101.87 ± 0.25		
200	5719			
400	9134			
400	9242	99.43 ± 1.02		
400	9276			
600	12815			
600	12837	99.58 ± 0.13		
600	12809			

*Average of three determinations

Robustness: The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2% **Table 6**.

System Suitability Parameters: System suitability parameters of Carbamazepine are summarised below in **Table 7**.

TABLE 6: ROBUSTNESS STUDY

S. no.	Parameters	Variation	Concentration (ng/band)	% RSD
1	Saturation Time	15 min ± 10 %	200	0.24
			400	0.08
			500	0.14
2	Time from application to development	(0, 30, 60, 90 min.)	200	1.16
			400	0.15
			500	0.19
3	Time from development to scanning	(0, 30, 60, 90 min.)	200	0.29
			400	0.65
			500	0.23
4	Scanning wavelength	284 ± 2 nm	200	1.39
			400	0.82
			500	0.36

TABLE 7: SYSTEM SUITABILITY PARAMETERS

Name	R_f	Concentration (ng/band)	Area	Asymmetry
Carbamazepine	0.78 ± 0.005	200	5735	1.20

Summary of Validation Study: The summary of validation parameters are summarised in **Table 8**.

TABLE 8: SUMMARY OF VALIDATION STUDY

S. no.		Validation Parameter	Result
1		Linearity	Y = 18.038x + 2042.7 R^2 = 0.9992
2		Range	100-600 ng/band
3		Assay	100.503 ± 0.947
		Precision	(%RSD)
4	A)	Intraday precision	0.3088
	A)	Interday precision	0.4287
		Accuracy	% Recovery
5		50%	100.72
		100%	99.40

	150%	100.67
6	LOD	15.079 ng/band
7	LOQ	45.696 ng/band
8	Specificity	Specific
9	Robustness	Robust

CONCLUSION: A new HPTLC method has been developed for the identification and quantification of CBZ. Low cost, faster speed, and satisfactory precision and accuracy are the main features of this method. Method was successfully validated as per ICH guidelines and statistical analysis proves that method is sensitive, specific, and repeatable. It can be conveniently employed for routine quality control analysis of CBZ as bulk drug in marketed tablets.

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CONFLICT OF INTEREST: The authors have no any conflict of interest.

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