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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CARBAMAZEPINE IN FORMULATED PRODUCT USING REVERSE PHASE ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY (RP-UPLC)

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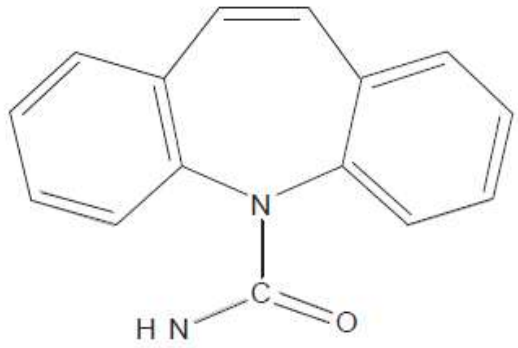
ABSTRACT
The novel method was performed to develop and validate a rapid and selective analytical method by using Reverse Phase Ultra performance Liquid Chromatography (RP-UPLC) technique for the analysis of carbamazepine in raw materials & their pharmaceutical dosage forms. The developed analytical UPLC method is superior in technology to conventional HPLC method with respect to speed, resolution, solvent consumption and cost of analysis. The compound was analyzed with a total run time of 2.5min. (In reverse phase) at 237 nm wavelength. Optimum retention was achieved on Waters Acquity UPLC BEH C18 column (2.1 × 30mm, 1.7µm) using gradient elution with mobile phase i.e. ACN: H_2O (50:50). The method showed excellent recoveries for all drugs in bulk. The developed UPLC method was validated with respect to specificity, linearity, precision, accuracy, ruggedness (reproducibility), robustness and stability. The method is economical in terms of the time taken and the amount of solvent used, thus promoting green chemistry concept. To the best of our knowledge, a work on method development and validation of Carbamazepine by using RP-UPLC technique, disclosed in this investigation, was not published elsewhere.

KEYWORDS: Carbamazepine, UPLC, Accuracy, Precision, Robustness.

INTRODUCTION
Carbamazepine (CBZ), 5-H-Dibenz [b.f] azepine-5-carboxomide (Fig.1), having empirical formula C15H12N2O and molecular weight is 236.26, is widely used as an anticonvulsant, antiepileptic and antimanic drug.[1,2] It is also used in trigeminal neuralgia (severe burning or stabbing pains in the face). CBZ was discovered in 1953 by chemist Walter Schindler at J.R. Geigy AG in Basel, Switzerland. The drug was then synthesized in 1960 by chemist Schindler. Later, its anti-epileptic properties were discovered. CBZ was first marketed as a drug to treat trigeminal neuralgia in 1962. It has been used as an anticonvulsant and antiepileptic in the UK since 1965, and has been approved in the US since 1974.In body it gets converted into its active metabolite carbamazepine-10, 11-epoxide which is having same activity as that of carbamazepine and it eliminates in the liver. [3] CBZ (5H-dibenzo[b, f]azepine-5-carboxamide) is insoluble in water, soluble in alcohol, acetonitrile and acetone. CBZ is available in market with the brand names Carbamazepen, Carbatrol, Carbazepine, Carbelan and Epitol. Although CBZ is poorly soluble in aqueous media, it has a high oral bioavailability in humans.[4] Various methods have been reported in the literature for the determination of CBZ, in particular those using chromatography.[5-11] Also, LC–mass spectrometry methods[12-15] have been reported for the detection of CBZ and its metabolites in aquatic environments and in plasma. Only one article that focused on forced degradation of CBZ used acid, base, oxidation, heat and photolytic conditions. Much degradation was not observed in CBZ samples under stress conditions like acid hydrolysis photolysis and thermal exposure. The earlier reported study of this drug was mainly performed by RP-HPLC methods on long columns with higher particle size, which were more time consuming. Even though the method was using complex mobile phase mixture with high flow rates, the analysis was lacking sensitivity and peak symmetry. The purpose of the present study is to develop a simple, sensitive, accurate, precise, rugged, and time saving method for the determination of Carbamazepine in formulated product. However there was some report available on the estimation of carbamazepine by UPLC method [16].The target is attained by selecting more advance technique of Waters Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7µm) which gives more accurate result in shorter run time. The developed method has been validated by

following several parameters as mentioned in ICH guideline [17-19] i.e. linearity, specificity, accuracy, precision, robustness, ruggedness, limit of detection etc.



Chemical structure of carbamazepine.

Fig.1 structure of carbamazepine

MATERIAL AND METHOD

Materials

Apparatus

Chemicals used in this study included gradient grade Acetonitrile (Sigma Aldrich, USA) and HPLC grade tri fluoro acetic acid (TFA) (Sigma Aldrich, USA). Water used for UPLC analysis was purified using Millipore Milli Q Plus water purification system (Millipore SAS, France).

Reagents and Chemicals

A well-characterized working standard of carbamazepine was procured from Jubilant Life Sciences Limited, India. Commercially available TEGRETAL (carbamazepine Tablet) purchased from local pharmacy (Noida, India) having batch number 159007 CP manufactured by Novartis Pharmaceuticals Ltd., India.

Methods

Solubility

From the literature review, carbamazepine is soluble in Acetonitrile and Water.

Selection of chromatographic method

Proper selection of the method depends upon the nature of the sample (ionic / ionisable / neutral molecule), its molecular weight and solubility. The drug selected in the present study is almost polar in nature and hence reversed phase or ion-pair or ion exchange chromatography method may be used. The reversed phase UPLC was selected for the separation because of its simplicity and suitability.

Selection of wavelength

The sensitivity of the any LC method which uses UV detection depends upon the proper selection of wavelength. An ideal wavelength is one that gives good response for all the drugs to be detected. During conditions optimization and from literature review we found that 237 nm is the appropriate wavelength for this analysis.

Chromatographic conditions

Chromatography separation was performed on Waters Acquity UPLC with photodiode array detector. The output signal was monitored and processed using masslynx software. The chromatographic column is Water Acquity BEH C18 column (50 × 2.1 mm, 1.7µm). The mobile phase of 0.1% TFA and acetonitrile in the ratio 80:20 v/v at a flow rate of 0.6 ml/min. The injection volume was 1.0µL and the chromatographic runtime of 2.5 min was used. A linear gradient elution method was applied as follows:

Time (min)	0.2	1.30	1.90	2.00	2.40	2.50
Flow (ml/min)	0.6	0.6	0.6	0.6	0.6	0.6
A%	90	90	55	35	10	99
B%	10	10	45	65	90	1

A- trifluro acetic acid B- Acetonitrile.

PREPARATION OF SOLUTIONS

Preparation of Buffer

Pipette out 1 ml of Tri Fluoro Acetic Acid in 1000 ml of milli Q water and sonicate for 1min and filter through 0.2µ 6, 6 Nylon membrane filter paper.

Preparation of Diluents

Mixed well Milli-Q water: Acetonitrile in a ratio (50:50) sonicated and degassed and filter through 0.2µ 6, 6 Nylon membrane filter paper.

Preparation of Standard Solution

Weighed accurately and transferred about 100.50 mg of CARBAMAZEPINE standard in a 100 ml volumetric flask. Pipette out 10 ml of this solution and volume made up to 20 ml, then further pipette out 10 ml of this solution and volume made up to 20 ml with diluent to dissolve, sonicated and degassed.

Preparation of Sample

Weighed and transferred drug substance 137.2 mg (equivalent to 100 mg of standard) in 100 ml volumetric flask. Pipette out 10 ml of this solution and volume made up to 20 ml, then further pipette out 10 ml of this solution and volume made upto 20 ml with diluent to dissolve, sonicated and degassed.

Assay procedure

Inject 1µL of the standard and sample solutions into the UPLC system and the chromatograms were recorded and measured the areas for the carbamazepine peak and calculate the % Assay by using following formula.

% Assay = (At/As) x (Ws/Ds) x (Dt/Wt) x (P/100) x(Avg. weight/Label Claim) x 100

Where,

At = average area counts of sample preparation,

As = average area counts of standard preparation

Ws= Weight of working standard taken in mg,

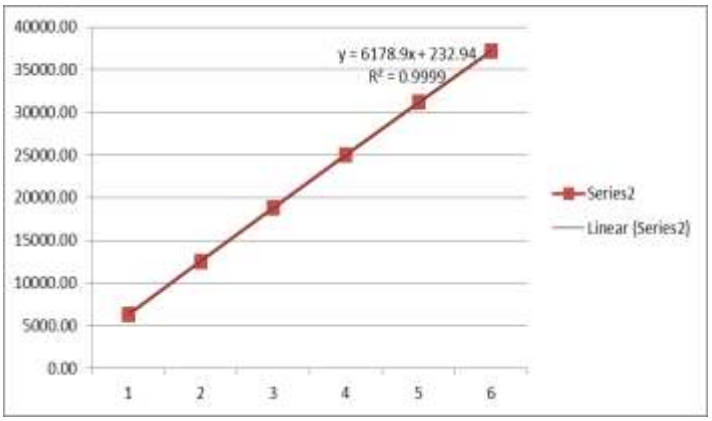
Wt =Weight of sample taken in mg
Dt = sample dilution
DS= standard dilution
P= Purity of Standard
In RP-UPLC method, chromatographic conditions were optimized to obtain, an adequate separation of eluted compounds with shorter run time, less consumptions of solvent and mass compatible for further studies.

Validation of developed UPLC method
Different chromatographic conditions such as mobile phase, wavelength, column and column temperature were experimented to achieve efficiency of the chromatographic system. Different gradients of buffer and solvents were checked in order to attain optimum retention of the API. Minimizations of run time and cost were the major tasks while developing the method. Based on International Conference on Harmonization (ICH) guidelines, the method was validated with regard to precision, specificity, reproducibility, accuracy, linearity, stability of solution, robustness, limit of detection and quantification.

Linearity
Linearity was assessed in the range of 25%, 50%, 75%, 100%, 125% and 150% of working concentration. Injections of all concentrations were carried out in replicate. Calibration curve was constructed by plotting the mean peak area versus concentration which was observed to be linear. The Linearity co-efficient of mean response which was plotted against respective concentration, was calculated. The results are summarized in Table-1 and Fig. 2.

Table 1 linearity data

Concentration (in %)	Peak Area		
	Injection-1	Injection-2	Average
25	6358.01	6270.61	63143.31
50	12543.95	12587.39	12565.67
75	18876.01	18871.54	18873.77
100	25026.91	25088.36	25057.63
125	31346.58	31021.36	31183.97
150	37277.5	37039.89	37158.69



Peak Area **% Conc.**
Fig. 2 calibration curve of carbamazepine

ACCURACY
Recovery of the assay method for carbamazepine was established by three determinations of test sample using Tablets at 50%, 100% and 150% concentration. Each solution was injected thrice (n=3) into UPLC system and the average peak area was calculated to obtain percentage recoveries. All the individual recoveries were found to be between 99.22% to 101.63%. All individual recovery levels were found to be within 0.09 to 0.24% (%RSD). The results are summarized in Table-2.

Table 2 Recovery studies of carbamazepine

Level	Sample area	Average area	Sample Wt. (mg)	Amount added (µg)	Amount recovered (µg)	% Recovery	Average % recovery	SD	% RSD
50%	12493.26	12483	69.50	**49.65**	50.86	101.71	**101.63**	0.10	**0.10**
	12469.39				50.76	101.52			
	12485.95				50.83	101.65			
100%	24054.48	24060	137.21	**98.03**	97.92	99.19	**99.22**	0.09	**0.09**
	24040.93				97.86	99.14			
	24085.29				98.04	99.32			

150%	37025.34	36998	208.60	**149.03**	150.72	100.43	**100.35**	0.24	**0.24**
	37068.42				150.89	150.89			
	36899.11				150.20	150.20			

Precision (system and method)

The precision of the system was evaluated by carrying out six independent injection of standard. The % RSD of peak area of the standard was found to be **0.18.** The results are summarized in Table 3.

Table 3 Result of system precision

S.no	Replicate	RT	Standard Area
1	Replicate-1	1.28	24268.85
2	Replicate-2	1.28	24383.94
3	Replicate-3	1.28	24311.92
4	Replicate-4	1.28	24375.35
5	Replicate-5	1.28	24342.40
6	Replicate-6	1.28	24354.18
	Average	**1.28**	24339.44
	SD	**0.0**	42.98
	%RSD	**0.0**	0.18

The precision of the method was evaluated by carrying out six independent injection of test sample against a qualified reference standard. The % RSD of peak area of the standard was found to be **0.33.** The results are summarized in Table 4

Table 4 Result of method precision

	Inj-1	Inj-2	Average area	RT
Sample-1	23986.38	24106.02	24046.20	1.28
Sample-2	23942.51	24018.80	23980.66	1.28
Sample-3	23897.27	23853.30	23875.29	1.28
Sample-4	24145.43	24038.71	24092.07	1.28
Sample-5	23967.30	24185.25	24076.28	1.28
Sample-6	23910.54	24154.53	24032.54	1.28
Average			24017.17	1.28
SD			79.56	0.00
%RSD			0.33	0.00

Reproducibility (Intermediate Precision)

An assay was performed by analyzing six samples of carbamazepine against qualified reference standard. The %RSD obtained from these samples was observed as **0.19** and %RSD of peak area of reference standard was observed as **0.18**. The results are summarized in Table 5.

Table 5 Result of intermediate precision

S.no	Name	Standard area	Test area		Avearge test area
			Injection 1	Injection 2	
1	Int.precision 1	24268.85	24267.59	24229.69	24248.64
2	Int.precision 2	24383.94	24399.00	24364.39	24381.70
3	Int.precision 3	24311.92	24213.21	24333.16	24273.19
4	Int.precision 4	24375.35	24217.20	24365.44	24291.32
5	Int.precision 5	24342.40	24283.68	24312.93	24298.31
6	Int.precision 6	24354.18	24247.93	24287.37	24267.65
	Average	24339.44			24293.47
	SD	42.98			46.69
	%RSD	0.18			0.19

Specificity

The specificity of the method was determined by comparing the chromatograms obtained from the sample containing carbamazepine standard stock with those of thetest sample. The specificity study reveals the absence of interference of impurities with the drug, since no extra peak appeared at the same retention time. The RSD for six replicate measurements of peak area of standard preparation was found to be **0.38**. The results are summarized in Table 6.

Table 6 Result of specificity

S.No.	RT (min)	STD Area	Test area
1	1.28	24924.88	24074.21
2	1.28	25125.74	24095.34
3	1.28	25007.68	
4	1.28	25082.04	
5	1.28	25218.46	
6	1.28	25027.93	
Mean	**1.28**	**25064.44**	**24084.775**
SD	**0.00**	**94.96**	**14.94**
% RSD	**0.00**	**0.38**	**0.06**

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. Such as change in flow rate (±0.10 mL/min), buffer concentration (±10%), column temperature (±5 ºC). In all the above varied conditions, the component of mobile phase was held constant, but no marked changes were observed in the chromatograms, which confirmed that the developed UPLC method is robust.

Limit of detection (LOD)

Limit of detection is the lowest amount of an analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The minimum concentration at which the analyte can be detected was determined by visual examination of signal to noise ratio which should be 3:1 with respect to height.

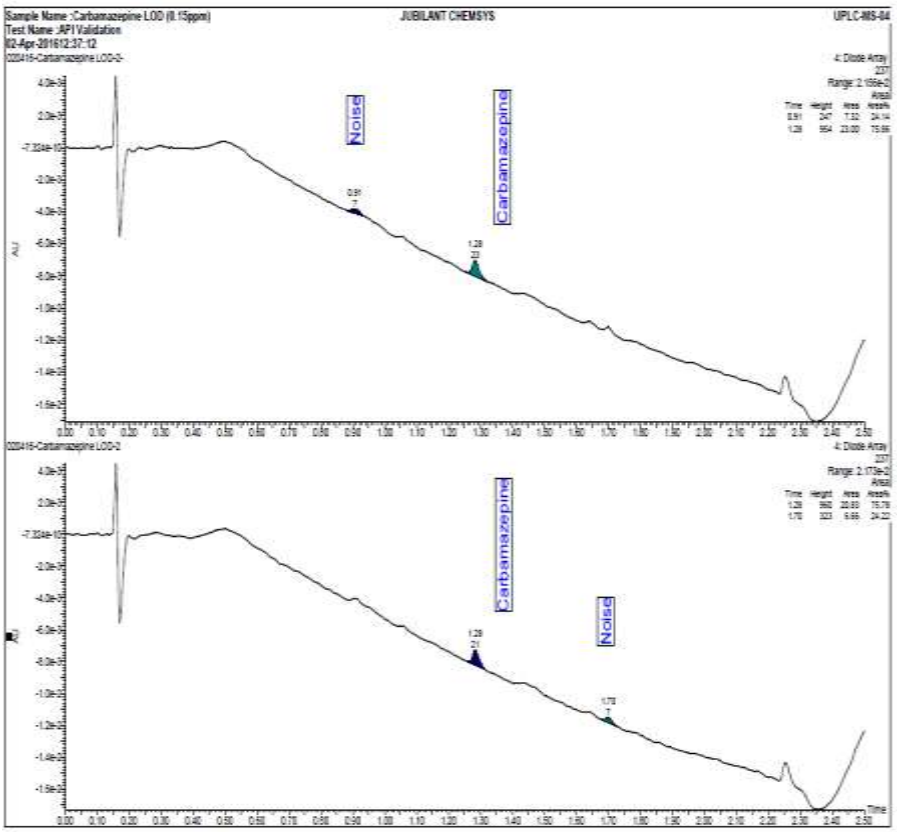


Fig.3 Chromatogram of LOD

CONCLUSION

A new method has developed to determine carbamazepine efficiently and accurately within a relatively short period by using Reverse phase UPLC-MS method. It showed a good precision (RSD<0.29%) and recovery (98.82% - 99.76%). and proved to be simple, linear, precise, accurate, robust, rugged and rapid. It gives faster elution, maintaining good separation more than that achieved with conventional HPLC. Short run time allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. This method can be directly used for LC-MS analysis on need basis.

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