Method Development and Validation by RP-HPLC Analysis for Quantitative Estimation of Carbamazepine

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Abstract: The present study aimed to develop and verify a high-performance liquid chromatography technique for measuring Carbamazepine in formulations that suggest stability. The Waters Alliance 510 with UV–484 Data Ace software (Instrument I.D.: AL-011) and the Agilent 1100 Series with Chromeleon software (Instrument I.D.: AL-013) are the two HPLC systems used in this procedure. Tetra hydro furan, methanol, and water were used to form a new mobile phase with a volume ratio of 30:120:850 (V/V/V) and a rate of flow of 2 ml/min. The detection was done at 230 nm. Stress testing was performed on Carbamazepine to assess the stability-indicating nature of the process. This encompassed degradation by hydrolysis in acidic, basic, and neutral environments, UV deterioration, and thermal deterioration. A linear relationship was discovered (r^2 = 0.999) for the concentration range of 50–150 ppm, which was further clarified by the regression equation Y=35.54x-254.8. Carbamazepine showed outstanding stability in conditions consisting of thermal, oxidative stress, acidity, base, and neutrality. The procedure's robustness, linearity, specificity, accuracy, and precision were all confirmed. The data demonstrate that it is suitable for commercial dosage form analysis in accordance with ICH guidelines since it is quick, accurate, precise, repeatable, and dependable.

Keywords: Carbamazepine, Forced Degradation studies, HPLC, ICH Guidelines, and Stability Studies.

Introduction

Neuropathic pain and epilepsy are the two primary conditions for which Carbamazepine is prescribed. It works for seizures just as well as phenytoin and valproate [1]. It is not effective in treating absence and myoclonic seizures. It can be taken in addition to other medications to treat schizophrenia and as a second line treatment for bipolar

illness. It is taken twice or four times a day. Preliminary data suggests that a formulation with controlled release has fewer side effects. Carbamazepine is treated with white circle pills. The chemical name for this compound is 5H-dibenzo [b, f] azepine-5-carboxamide. The molecular weight is 236.26, and the formula is $C_{15}H_{12}N_2O$[2]. Neither 95.0% nor 105.0% of the prescribed dosage is exceeded by the test limits for the 200 mg Carbamazepine tablets. An overview of the results from the HPLC method verification for the assay of Carbamazepine in 200 mg tablets is given in this report on analytical method verification, which also highlights the verification activity conducted on the 200 mg tablets of the drug.

The current effort is concentrated on creating an analytical method for consistently and concurrently figuring out Carbamazepine (CBZ) quantification[3]. Several methods have been reported for the determination of CBZ standards and in pharmaceutical formulations. These include spectrophotometric methods[4-7], spectro fluorimetry methods [8, 9], gas-liquid chromatography (GC) [10, 11], FT-Raman spectroscopy[12], and liquid chromatography[13-17]. Our objective was to create an affordable, user-friendly, repeatable, fast, accurate, and dependable HPLC method for determining CBZ in pharmaceutical formulations. To show that this tried-and-true method can be applied to genuine samples. In general, CBZ is helpful in treating various types of seizures. Internal standards CBZ and CPZ are in use; their structure is depicted in Figure 1 below. The word "CBZ" also refers to a non-polar, acidic aromatic ester.

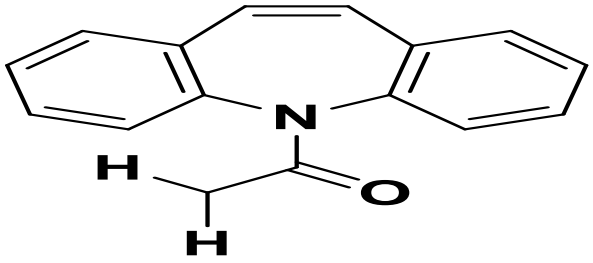


Fig. 1: Chemical structure of Carbamazepine

An analysis of the literature reveals that there has only been one previous method that addresses stability and provides methods for identifying CBMZ[18]; however, this methodology has certain drawbacks, such as an unduly lengthy separation time (14 minutes) and limited sensitivity. The goal of this work is to ascertain the CBMZ content of bulk powder and tablets by means of a novel, inexpensive, sensitive, simple, accurate, exact, and quick stability indicating fully validated chromatographic technique employing isocratic mode. Developing and validating a rapid, dependable, and cost-effective reverse phase high-performance liquid chromatography (RP-HPLC) method for measuring Carbamazepine in bulk materials and tablet formulations was the primary objective of

this work. In accordance with the guidelines provided by ICH Q1A (R2), stability testing was done to gather information on how the quality of the drug substance or drug product changes over time when exposed to various environmental factors such temperature, humidity, and light. The ultimate goal of this testing is to establish a shelf life or retest time for the drug product, along with recommended storage conditions.The current investigation aimed to validate the analytical protocol for measuring the amount of Carbamazepine in 200 mg Carbamazepine tablets using Reverse Phase High-Performance Liquid Chromatography (HPLC) in compliance with the recommended analytical method.

Materials and Methods

The analytical column used in the analysis is an HPLC column known as Nucleosil 10 CN. It is composed of packing material and has dimensions of 25 cm by 4.6 mm by 10µm in diameter. Two HPLC systems are utilized: the Agilent 1100 Series with Chromeleon software (Instrument I.D.: AL-013) and the Waters Alliance 510 with UV–484 Data Ace software (Instrument I.D.: AL-011). The equipment required for quantitative analysis includes a UV/VIS detector. The necessary amounts comprise the following: Carbamazepine working standard; 200 mg of Carbamazepine tablets; Tetra hydro furan (AR grade); formic acid (AR grade); tri ethylamine (AR grade); methanol (HPLC grade); and water (HPLC grade). The analytical configuration allows for precise analysis, while the UV–484 Data Ace and Chromeleon software facilitate data processing for both HPLC systems.

Analytical method: The quantitative determination is carried out by HPLC system equipped with UV/VIS detector.

Chromatographic conditions:

Column	**:**	Nucleosil 10 CN - 25 cm × 4.6 mm x 10-µm
Mobile Phase	**:**	30 volumes of tetra hydro furan, 120 volumes of methanol and 850 volumes of water, adding 0.2 volumes of anhydrous formic acid and 0.5 volumes of tri ethylamine to 1000 volumes of the solution. Mix well. Filter through 0.2 µm Nylon membrane filter paper and degas prior to use.
Wavelength	**:**	230 nm
Flow Rate	:	2.0 ml / minute
Injection volume	:	20 µl
Run time	:	15 minutes
Blank solution	:	Use Diluent as blank
Diluent	:	Methanol and Water 50:50

Preparation of placebo solution: Accurately measure out forty-five milligrams of Carbamazepine placebo and transfer it to a 20 milliliter volumetric flask. After that, sonicate to dissolve the 10 ml of diluent that was added. Next, use the diluent to dilute the solution to volume. Lastly, transfer 1 ml of the solution, dilute it, and stir it in a 10 ml volumetric flask. Lastly, pass it through a nylon membrane filter with a 0.2 µm pore size.

Preparation of Carbamazepine standard solution: Precisely weigh out twenty milligrams of the working standard Carbamazepine, and then transfer the contents into a volumetric flask measuring twenty milliliters. In 10 milliliters of diluent, sonicate to dissolve. Utilizing a diluent, dilute to volume. It is necessary to transfer, dilute, and mix 1 milliliter of the solution into a 10 milliliter volumetric flask. Pass the mixture through a 0.2µm-pore-size nylon membrane filter.

Preparation of test solution: Determine the mean weight of ten tablets. It was in powder form. Accurately weigh out the sample of Carbamazepine powder to a twenty milliliter capacity. To dissolve, add ten milliliters of diluent and sonicate. Use a diluent to dilute to volume. The next step is to transfer, dilute, and mix 1 milliliter of the solution into a 10-milliliter volumetric flask. Pass the mixture through a nylon membrane filter with a pore size of 0.2µm. A tablet weighs 245 milligrams on average. **Limits:** The number

of theoretical plates cannot be fewer than 2000. There should be less than 2.0 for the tailing factor. The percentage RSD must not be higher than 2.0%.

System suitability solution: As a system suitability solution, use the standard working solution for Carbamazepine.

Procedure: The working standard solution for Carbamazepine, known as the system suitability solution, should be injected five times in identical amounts. After that, inject two times with the test solution and record the chromatograms. If a peak in the test solution appears blank, dismiss it. After five injections of the system suitability solution (the standard working solution for Carbamazepine), calculate the % RSD. Examine the tailing factor and theoretical plates of the peak in the chromatogram that was obtained after the sixth injection of the system suitability solution (working standard solution for Carbamazepine). These are the boundaries. There must be a minimum of 2000 hypothetical plates. A tailing factor of less than 2.0% is desirable. There should be no more than 2.0% RSD.

Results and Discussion

ICH guidelines The HPLC process is assessed using quality themes to make sure it complies with validation parameters[19]. In particular, ICH Guideline numbers Q2A and Q2B of CPMP/ICH/281/95. System suitability criteria were carefully monitored throughout the validation research and the results are painstakingly reported in the validation report. An overview of the validation data can be seen here.

Specificity/selectivity: Injecting the diluent blank solution, excipient mix, system suitability solution, and test solution allowed for selectivity. The requirements for approval were as follows: The Carbamazepine peak needs to be clearly identified from every other peak and from each other. The diluent blank solution and excipient mix solution shouldn't have any peaks at the Carbamazepine retention time. By using the analytical method, it was determined that the system suitability criteria satisfied the pre-specified acceptance conditions.

Table 1. System Suitability - Selectivity

Sr. No.	Area of Carbamazepine
1	3023.81
2	3037.52
3	3045.71
4	3050.52

5	3062.13
Mean	3043.94
Standard deviation (±)	14.35
(%) Relative standard deviation	0.47

The method's specified wavelength was used to process each injection. The placebo with Carbamazepine peak and diluent blank solution did not interfere in any way. This highlights the selectivity of the technique. The relative standard percentage was clearly less than one (i.e., 0.47), as shown in Table 1. The new method just requires simple sample preparation techniques and permutations of mobile phases. It is both selective and rapid (10.15 min run time). The developed method produced end data that was satisfactory and selected.

Forced degradation: In order to determine whether the assay method is stable and to track down any deteriorated substances, forced degradation studies are carried out. As indicated in Table 2, Carbamazepine WS and sample are stressed using 5N HCl, 5N NaOH, thermal degradation, and UV degradation. Every one of the aforementioned solutions has been chromatographed and chromatogram med. For degradation, the ensuing stress conditions are adhered to. Table 3 displays the results of the suggested approach for system applicability of forced degradation. It is noted that the mean area is 2969.69 and the percentage RSD is 0.48. Tables 3 and 4 provide a detailed summary of the deterioration results under the given stress conditions based on the collected data.

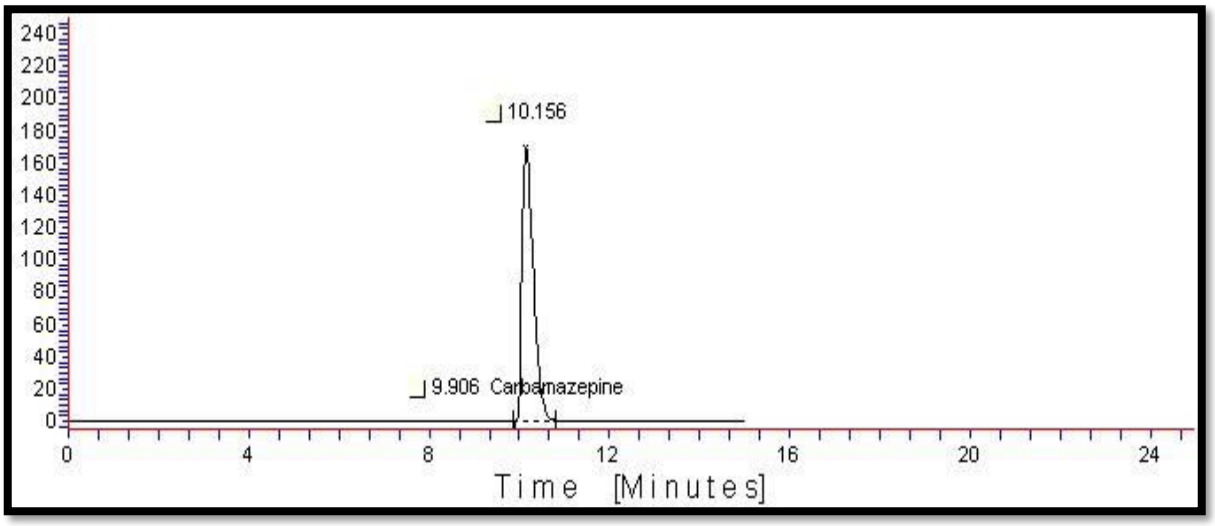
Table 2. Conditions for Forced Degradation

Sample Stress condition	Description of Stress Condition
Acid degradation	5N HCl heated at about 60°C for 10 min on a water bath.
Alkali degradation	5N NaOH heated at about 60°C for 10 min on a water bath.
Thermal degradation	105ºC for 12 hours
UV degradation	expose to UV-radiation for 7 days

Table 3. System Suitability– Forced Degradation

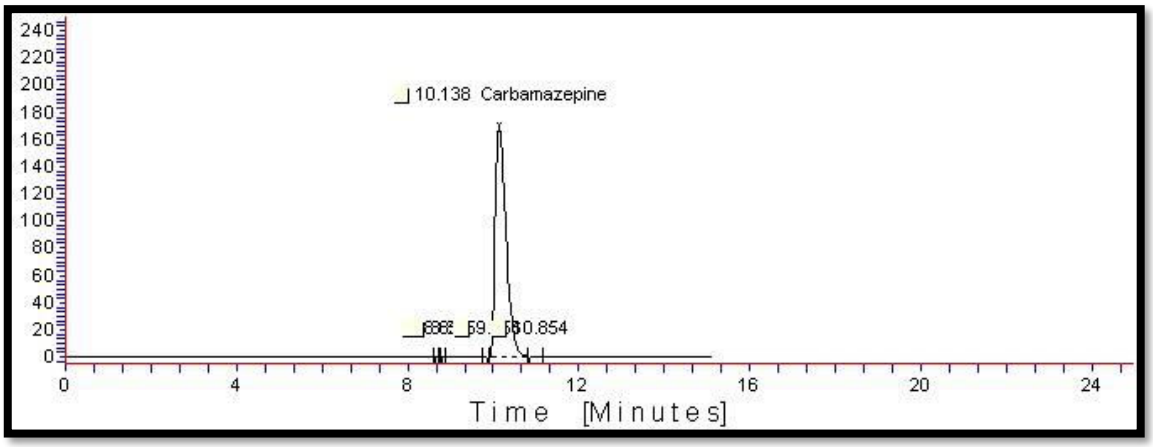
S. No.	Area of Carbamazepine
1	2987.03
2	2976.48
3	2967.72

4	2969.24
5	2947.98
Mean	2969.69
Standard deviation (±)	14.34
(%) Relative standard deviation	0.48



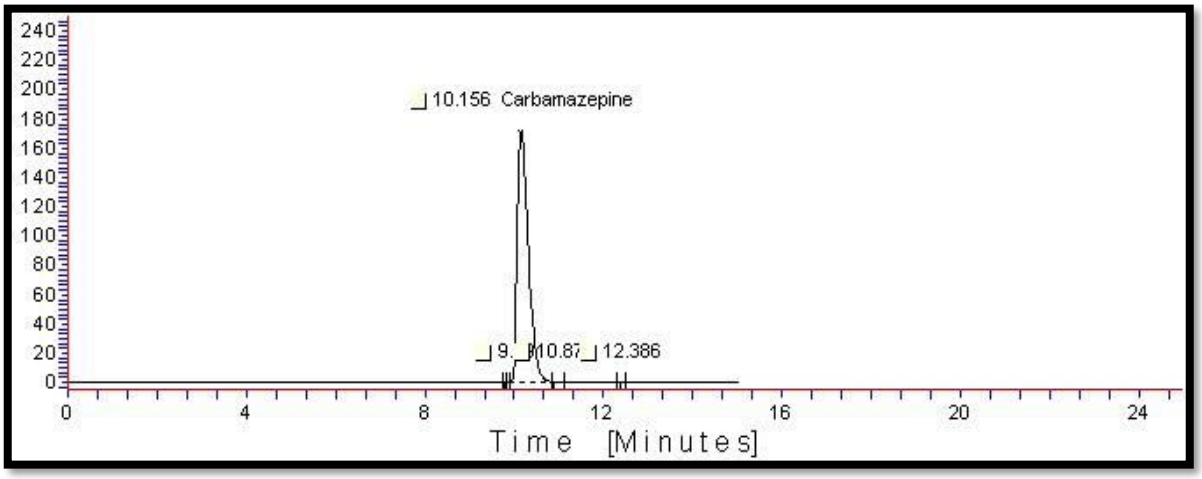
Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	9.906	0.241	0.078	0.008	0.046	0.05
2	10.156	3054.947	170.81	99.992	99.954	0.3
Total		**3055.188**	**170.888**	**100**	**100**	

Fig. 2: Chromatogram of Carbamazepine sample in acid degradation



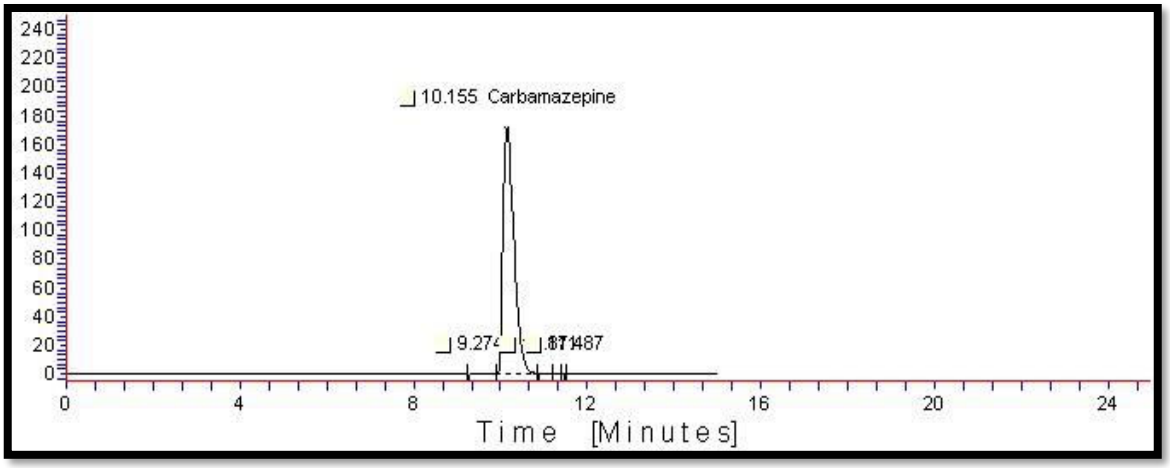
Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	8.658	0.364	0.08	0.012	0.046	0.083
2	8.825	0.137	0.032	0.004	0.018	0.083
3	9.856	0.615	0.093	0.02	0.054	0.116
4	10.139	3069.648	172.043	99.777	99.519	0.3
5	10.854	5.749	0.626	0.187	0.362	0.166
Total		**3076.513**	**172.874**	**100**	**100**	

Fig.3: Chromatogram of Carbamazepine sample in alkali degradation



Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	9.79	0.114	0.038	0.004	0.022	0.05
2	10.156	2975.912	172.895	99.841	99.655	0.283
3	10.872	4.01	0.481	0.135	0.278	0.15
4	12.386	0.61	0.078	0.02	0.045	0.15
Total		**2980.646**	**173.492**	**100**	**100**	

Fig. 4: Chromatogram of Carbamazepine sample in thermal degradation



Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	9.274	0	0	0	0	0.033
2	10.156	3071.843	172.961	99.834	99.66	0.3
3	10.871	4.769	0.534	0.155	0.308	0.15
4	11.487	0.323	0.055	0.011	0.032	0.1
Total		**3076.935**	**173.55**	**100**	**100**	

Fig. 5: Chromatogram of Carbamazepine sample in UV degradation

Table 4. % of Degradation by Applying Different Conditions

Acid Stress	**% Degradation**
Standard	0.169
Sample	0.008
Alkali Stress	**% Degradation**
Standard	0.181
Sample	0.223
Thermal Stress	**% Degradation**
Standard	0.164
Sample	0.159
UV Stress	**% Degradation**
Standard	NA
Sample	0.166

The degradation peaks ought to be separated by a fair amount. The purity of the Carbamazepine peak ought to pass. There is no evidence of peak-to-peak interference between the chromatograms of the degradation preparations, as shown in Figures 2–5. When degradation is driven, the deterioration peaks separate from one another. The

Carbamazepine peak's purity is decreasing. Consequently, the method is stable and extremely accurate, selective, and specific for measuring Carbamazepine in Carbamazepine Tablets 200 mg by HPLC since the breakdown products are distinct from both Carbamazepine and each of the surrounding peaks.

Linearity

Linearity and range assessment for sample: In order to evaluate the linearity of Carbamazepine, five sample solutions with concentrations ranging from 50% to 150% of the theoretical assay preparation concentration were made. The process that was specified was followed in injecting both the linearity solution and the system suitability solution into the system. The correlation coefficient was calculated and a graph was made to display the concentration vs. peak response. To be approved, this evaluation needed a correlation coefficient of 0.999.

Results: Table 4.5 lists the order of injections for linearity. The standard deviation and linearity of Carbamazepine were computed and reported; the five test solutions' percentage RSD is 0.37. Table 5 makes it clear that Carbamazepine has a mean area of 3178.80. The analytical method's predetermined acceptance standards were successfully fulfilled, indicating that the system appropriateness conditions were met. (For results regarding system appropriateness, please see Table 6).

Table 5. System Suitability - Linearity of Sample

Sr. No.	Area of Carbamazepine
1	3196.50
2	3165.12
3	3172.74
4	3178.13
5	3181.51
Mean	3178.80
Standard deviation (±)	11.67
(%) Relative standard deviation	0.37

Plotting the values of the average peak area against the sample concentration expressed as a percentage allowed for the construction of a linearity graph, which

corresponded to different quantities of Carbamazepine. Table 6 displays the findings from the linearity investigation. Strong evidence of a relationship between peak area and analyte concentration was found[20].

Table 6. Results for Linearity of Sample

Linearity Level	Sample Concentration (in %)	Sample Concentration(in ppm)	Peak Area	Correlation Coefficient
Level – 1	50	50	1533.25	**0.999**
Level – 2	75	75	2445.08	
Level – 3	100	100	3237.97	
Level – 4	125	125	4162.32	
Level – 5	150	150	5117.17	

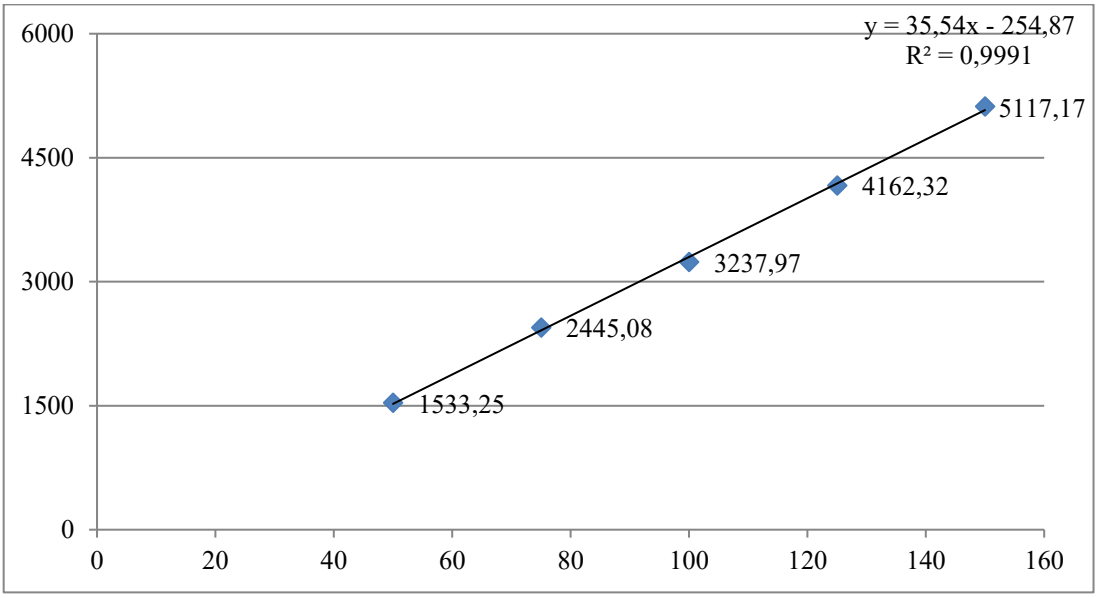
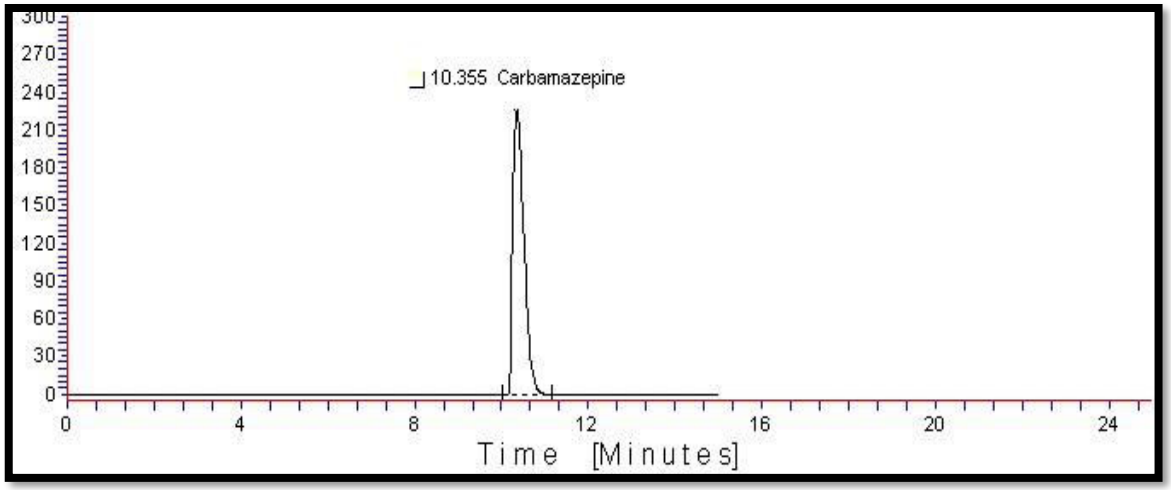


Fig.6: Linearity graph of Carbamazepine standard



Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	10.356	4179.04	226.436	100	100	0.299
Total		**4179.04**	**226.436**	**100**	**100**	

Fig. 7: Chromatogram of Carbamazepine sample

On a linearity graph, which was shown to be a straight-line graph in Figure 6, the average area at each level was plotted against the concentration (%). A R^2 value of 0.999 and a value of Y=35.54x-254.8 were found in the calibration curve regression equation. Higher than 0.999 was found to be the correlation coefficient. Table 6 displays the findings about the Carbamazepine sample's linearity. 50–150 ppm was the range covered by the analytical procedure. Consequently, it was found that the process was linear from 50% to 150% of the operating concentration. A peak was found at 10.355 retention time in the chromatogram of the Carbamazepine sample, as can be shown in Figure 7. After being determined to be below acceptable limits.

Precision assessment and method precision evaluation

Procedure: The analytical process was followed in the preparation of six test solutions containing 200 mg of Carbamazepine. The assay percentage RSD for six test solutions was calculated. Table 7 below displays the findings from tests conducted on five samples of analyte solutions using analyst-1. The table shows that the mean area is 2958.65 and the % RSD is 0.21. Table 8 displays the percent relative standard deviation (% RSD) that was obtained from the assay of these six test solutions. The six test solution findings' RSD shouldn't be more than 2.0%. The system appropriateness criterion was found to satisfy the pre-established acceptance conditions using the analytical method. Table 8 displays the assay findings from the six test solution formulations.

Table 7. System Suitability - Method Precision

Analyst – 1 HPLC No.: EH/R&D/HPLC-024

Sr. No.	Area of Carbamazepine
1	2952.26
2	2964.35
3	2952.62
4	2965.71
5	2958.29
Mean	2958.65
Standard deviation (±)	6.32
(%) Relative standard deviation	0.21

Table 8. Results of Method Precision

Test Solution	% Assay of
1	100.76
2	101.83
3	99.95
4	98.87
5	98.58
6	99.82
Mean	99.97
Standard deviation (±)	1.20
(%) Relative standard deviation	1.20

Table 4. 8 indicates a mean area of 99.97 square feet and an RSD of 1.20 percent. The RSD of the findings of the Carbamazepine assay utilizing six test solutions detreminatins is rather small.

Intermediate precision

Procedure: Six test solutions totaling 200 mg of Carbamazepine tablets were prepared on different days using the analytical method. Another analyst analyzed these test solutions using a different HPLC apparatus and column (of the same make and model, but different serial numbers). For a total of twelve test solutions, the percent RSD of the assay results was calculated using six samples from method precision and six samples from intermediate precision.

Acceptance criteria: Twelve test solutions totaling six with method precision and six with intermediate accuracy should have a percent RSD of no more than 2.0%. The analytical method was used to determine whether the system suitability criteria satisfied the pre-established acceptance standards[21]. For information on system appropriateness, see Table 9. Table 10 displays the test results from six test solutions. Table 11 displays the assay findings' percentage RSD based on procedure precision and intermediate accuracy (12 total outcomes). The percent RSD of twelve test solutions six from method precision and six from intermediate precision was calculated.

Table 9. System Suitability - Intermediate Precision

Analyst – 2 HPLC No.: EH/R&D/HPLC-023

Sr. No.	Area of Carbamazepine
1	2865.97
2	2876.85
3	2868.42
4	2874.68
5	2888.42
Mean	2874.87
Standard deviation (±)	8.78
(%) Relative standard deviation	0.31

Table 10. Results of Intermediate Precision

Test Solution	% Assay of Carbamazepine
1	101.08
2	100.15
3	102.86
4	100.50
5	98.53
6	100.17
Mean	100.55

Standard deviation (±)	1.41
(%) Relative standard deviation	1.41

Table 11. Results of Twelve Test solutions of Carbamazepine in (Six of Method Precision & Six of Intermediate Precision)

Analysis performed during method precision study By Analyst 1 on system 1 and on column 1 on day 1	
Same column	**% Assay of Carbamazepine**
1	100.76
2	101.83
3	99.95
4	98.87
5	98.58
6	99.83
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Column sr. no.	**015322030142 01**
Test Solution	**% Assay of Carbamazepine**
7	101.08
8	100.15
9	102.86
10	100.50
11	98.53
12	100.17
Mean of twelve	100.26
Standard	1.29
(%)R. Std.	1.29

The fact that the data's percent RSD values were substantially less than 2% suggests that the approach was adequately accurate, according to the findings. On two separate days, two different analyzers analyzed six test solutions from the same lot of the pharmaceutical product using two distinct pieces of equipment at the same facility and two different columns of the same make but different serial numbers. The twelve assay findings, six from intermediate precision and six from procedure precision, are found to have a percentage RSD of less than 2.0%. As a result, the technique's accuracy and dependability are proven.

Accuracy assessment methodology

Procedure: Analyzing Carbamazepine test solutions which were made by combining Carbamazepine API with a variety of excipients was how the accuracy analysis was carried out. Three concentration solutions were created by adding a precise amount of Carbamazepine API to the excipient blend. These solutions equated to 50%, 75%, 100%, 125%, and 150% of the test concentration. It takes a mean recovery of between 98.0% and 102.0% for any concentration level.

Results: Using the analytical method, it was determined that the system suitability criteria satisfied the pre-established acceptance norms. To view the system suitability result, see Table 12. Table 13 presents the findings of the accuracy investigation.

Table 12. System Suitability - Accuracy (% Recovery)

Sr. No.	Area of Carbamazepine
1	3137.65
2	3120.68
3	3134.57
4	3123.24
5	3148.73
Mean	3132.97
Standard deviation (±)	11.39
(%) Relative std. deviation	0.36

Table 13. Accuracy (% Recovery) – Results

Level of addition	Amount of Carbamazepine added in mg	Amount of Carbamazepine found in mg	Recovery (%)
First Level (Rec-50 %)	10.1	9.97	98.71
Second Level (Rec-75 %)	15.5	15.41	99.42
Third Level (Rec-100 %)	20.7	20.92	101.06
Fourth Level (Rec-125 %)	25.8	26.06	101.01
Fifth Level (Rec-150 %)	31.7	32.03	101.04
Mean			100.25
Standard deviation (±)			1.11
(%) Relative standard deviation			1.11

Remarks: The percentage recovery for Carbamazepine varies from 98.0% to 102.0 % at each level. For each recovery level, the RSD is less than 2.0%. The pre-established acceptance criteria for the recovery investigation are satisfied by the protocol-compliant analytical method. As a result, the process' correctness is determined.

Acceptance criteria: According to the analytical technique, the system suitability criteria should be satisfied, and the percentage RSD that is different between the average method precision result and the results generated under changing conditions shouldn't be more than 2.0%.

As a result, the analytical technique complies with the protocol's specified acceptance criteria for the recovery research. The repeatability is within the range of 1.2 and the mean percentage of drug recovery is 100.25. The percentage recovery results demonstrate the efficacy of the recommended strategy for Carbamazepine and are consistent with other reports of drug analysis for Valsartan[22]. In conclusion, the accuracy of the procedure is confirmed. In order to achieve acceptance standards, the method must also meet system appropriateness requirements, and the percentage RSD between results produced under modified conditions and the method's average precision cannot be greater than 2.0%. As a result, we can declare that the suggested approach is precise.

Robustness study

Experimental procedure: We prepared two test solutions for this robustness investigation. Prepare two test solutions of the same lot of Carbamazepine in 200 mg Carbamazepine tablets in accordance with the analytical procedure. These test answers were created using the given analytical procedure. These test solutions were injected

under the different chromatographic settings listed below, together with a diluent blank solution and a system-suitability solution: Variations in column lot, wavelength (± 2 nm), flow rate (± 0.2 ml/minute), and mobile phase composition (± 0.2).

Change in column lot: Under standard experimental conditions, the dimensions of the 10 CN Nucleosil columns are 25 cm × 4.6 mm x 10-µm. We evaluated throughout this experimentation if the system suitability requirements satisfied the predefined acceptance criteria as stated in the analytical procedure. See Table 14 for specific results related to system appropriateness.

Table 14. System Suitability - Robustness with Change in Column

Sr. No.	Area of Carbamazepine	
	Same column	Different column
1	2940.43	2948.79
2	2958.65	2955.56
Mean	2949.54	2952.18
Standard Deviation (±)	12.88	4.78
(%) Relative Standard Deviation	0.44	0.16

Table 15 presents the assay findings obtained under varying flow rate circumstances.

Table 15. Results for Change in Column

Flow rate →	Same column	Different column
Sample	% Assay	
Test solution	100.65	100.83
Avg. assay result from method precision	99.97	99.97
Mean	100.61	100.40
Standard Deviation (±)	0.48	0.61
(%) Relative Standard Deviation	0.48	0.61

Change in flow rate (± 0.2 mL/minute) (Normal experimental condition: 2.0ml/minute)**:**

The system suitability assessment confirmed conformance to the predefined acceptance criteria in accordance with the analytical procedure. For comprehensive results on system appropriateness, please refer to Table 16.

Table 16. System Suitability - Robustness with Change in Flow Rate

Sr. No.	Area of Carbamazepine	
	1.8 mL/minute	2.2 mL/minute
1	3112.52	3129.93
2	3119.79	3134.72
Mean	3116.15	3132.33
Standard Deviation (±)	5.14	3.39
(%) Relative Standard Deviation	0.16	0.11

Table 17 presents the assay findings obtained under varying flow rate circumstances.

Table 17. Results for Change in Flow Rate

Flow rate →	1.8 mL/minute	2.2mL/minute
Sample	% Assay	
Test solution	100.47	100.88
Avg. assay result from method precision	99.97	99.97
Mean	100.22	100.43
Standard Deviation (±)	0.35	0.64
(%) Relative Standard Deviation	0.35	0.64

Change in wavelength (± 2 nm) (Normal experimental condition: 230nm):

According to the analytical procedure, the system suitability evaluation showed compliance to the predetermined acceptance criteria. For specific results on system appropriateness, please refer to Table 18.

Table 18. System Suitability - Robustness with Change in Wavelength

Sr. No.	Area of Carbamazepine	
	228 nm	232 nm
1	3125.00	3169.45
2	3133.51	3159.78
Mean	3129.25	3164.61
Standard Deviation (±)	6.02	6.84
(%) R. Standard Deviation	0.19	0.22

The presented table designated. Table 19, shows the assay findings obtained under different wavelength circumstances.

Table 19. Results for Change in Wavelength

Wavelength →	228 nm	232 nm
Sample	% Assay	
Test solution	100.92	100.38
Average assay result from	99.97	99.97
Mean	100.45	100.18
Standard Deviation (±)	0.67	0.29
(%) Relative Standard Deviation	0.67	0.29

Change in composition of mobile phase: Under typical experimental conditions, Tetra hydro furan: Methanol: Water = 30:120:850 v/v makes up the solvent composition. The predetermined acceptance criteria described in the analytical procedure were found to be in line with the system suitability characteristics. For the comprehensive results of system appropriateness, please see Table 20.

Table 20. System Suitability - Robustness with Change in Mobile Phase Composition

Sr. No.	Area of Carbamazepine	
	T	T
1	2947.03	2947.06
2	2956.48	2954.67
Mean	2951.75	2950.86
Standard Deviation (±)	6.68	5.38
(%) R S D	0.23	0.18

Table 21 presents the test results obtained with a modification in the composition of the mobile phase.

Table 21: Results for Change in Composition of Mobile Phase

Mobile phase composition	T 29:A119:W852	T 31:A121:W848
Sample	**% Assay**	
Test solution	101.44	101.85
Average assay result from method precision	99.97	99.97
Mean	100.71	100.91
Standard Deviation (±)	1.04	1.33
(%) Relative Standard Deviation	1.03	1.32

Different conditions related to the column lot, flow rate, wavelength, and mobile phase makeup were examined for the same lot of 200 mg Carbamazepine tablets. All system suitability tests passed with the predetermined standards, and the percentage difference (% RSD) between the average method precision result and the results obtained under modified conditions was less than 2.0%. The protocol-compliant analytical approach satisfies the predetermined acceptance criteria for the robustness investigation. As such, it is confirmed that the procedure is resilient.

Stability of analytical solution

Experimental procedure: On the 0^{th}, 12^{th}, 24^{th}, 36^{th}, and 48^{th} hour of the experiment, the 200 mg Carbamazepine tablet test solution and the system suitability solution were prepared. These solutions were then stored for a maximum of 48 hours at a time at room

temperature. After that time, the solutions were analyzed using a newly created test solution. On the day of analysis, a new answer for system suitability was created. The 200 mg Carbamazepine tablets in the sample were tested using a calculation. If there is no appreciable variance in the percentage assay, the analyte is deemed stable. For a maximum of two days, the produced solution was injected sporadically into the chromatographic system to test the stability of the reference solution. The results of the experiment on solution stability are shown in Table 22.

Table 22: Results for Solution Stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Carbamazepine**
0^{th}hr	99.40
12^{th}hr	99.66
24^{th} hr	99.78
36^{th} hr	100.01
48^{th} hr	99.50
Mean	99.67
Standard deviation (±)	0.24
(%) Relative standard deviation	0.24

The mean average area of 99.67 and the percent RSD of 0.24, as presented in Table 22, are both

At room temperature, there was no appreciable shift in the assay level for the test solution for up to 48 hours. Consequently, it may be concluded that the solution remains stable for a maximum of 48 hours at room temperature.

Conclusions

Overall, we have successfully developed and validated an HPLC approach for the quantitative evaluation of Carbamazepine in its different forms in compliance with ICH standards. The drug's HPLC chromatogram was used to calculate the average retention length of Carbamazepine, which came out to be 10.355 minutes. The validation trials conducted have demonstrated the good attributes of the approach, including its speed, simplicity, accuracy, precision, specificity, selectivity, and cost-effectiveness. The degradation peaks exhibit a distinct separation from one another when degradation is

imposed. It is becoming less pure at the Carbamazepine peak. Due to the fact that the deteriorated products may be easily distinguished from both Carbamazepine and every peak in the vicinity. It was discovered that this method's linearity fell between 50% and 150% of the working concentration. The range of the analytical procedure is 50 ppm to 150 ppm.

The accuracy and precision of the approach were within acceptable limits. It's believed that the HPLC technology of today is appropriate. It was discovered that the analytical solution remained stable at room temperature for 48 hours. It was decided that each recovery level had a percentage RSD of less than 2.0% and that the range of recovery levels for Carbamazepine is always between 98.0% and 102.0 %. The mean average recovery percentage was 100.25. This technique is useful for assessing samples taken during quick stability testing since it can successfully separate the medication from its breakdown products as well as any related compounds and excipients present in tablet formulations. These factors lead to the conclusion that the analytical approach was determined to be suitable for both routine analysis and stability research after it was validated. As a result, both quantitative quality control and upcoming pharmaceutical research may benefit from the suggested technique. Ultimately, the suggested methodology makes sense and is practical for analyzing drugs in large quantities.

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