# Spectrophotometric and High Performance Liquid Chromatographic Determination of Carbamazepine in Tablets Dosage Form

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

*Original Research Article*

## ABSTRACT

Today, millions of people suffer from epilepsy, one of the most common chronic neurological diseases worldwide. Carbamazepine is a first-line drug used in the treatment of epilepsy. High performance liquid chromatographic and spectrophotometric methods have been developed for the determination of carbamazepine in tablet dosage forms. UV spectrums were recorded in the wavelength range of 200-800 nm using methanol solvent, and the wavelength for determining carbamazepine was selected as 286 nm. LC analysis was performed using Agilent Extend-C18 column and mobile phase composed of $KH_2PO_4$ solution(pH: 3.5) and acetonitrile (40:60 v/v) at a flow rate of 1.2 mlmin$^{-1}$. These analytical methods were validated in agreement with the International Conference on Harmonization (ICH) guidelines using the following analytical parameters: specificity, linearity, precision, accuracy, detection and quantification limits, and robustnes. Analytical methods showed wonderful linearity ($r^2$>0.999) in the concentration range of 5-25 µg mL$^{-1}$ for boths methods. Precision (R.S.D%<1.17) and recevery for both methods was in the range of 99-101%, which shows accuracy of these methods. These proposed methods were found to be accurate, reliable, fast, simple, The F-test and t-test were used to perform statistical

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comparison of these methods, and the results of both analytical methods indicated no significant difference. As a result, the proposed methods can be used to analyze carbamazepine in pharmaceutical formulations.

## 1. INTRODUCTION

Epilepsy is a chronic neurological disorderand it affects millions of individuals all over the World [1–3]. Recurrent unprovoked seizures is a symptom of epilepsy and the primary objective of epilepsy therapy is to achieve total seizure independence while avoiding negative side effects. Continuous antiepileptic action is necessary for this, which can only be achieved if drug concentrations in the plasma and brain stay at therapeutic levels [4].

The incidence of epilepsy is higher in developing countries due to limited health services such as malnutrition, birth and head trauma [5,6]. Epileptic seizures are controlled by various methods such as surgical treatment, ketogenic diet, vagal stimulation and most preferred antiepileptic drugs therapy that reduces seizure frequency and severity. The antiepileptic drugs therapy to be used in treatment (conventional/ classical, second generation) should be effective, long term preservative, well tolerated and increase the quality of life of the patient [5,7]. Appropriate pharmacological treatment should begin with a single drug called monotherapy, taking into account the epilepsy syndrome and the type of seizure. If monotherapy fails due to lack of efficacy, polytherapy as a combination of different antiepileptic drugs may be considered for the treatment of epilepsy with additive/synergistic effect of drugs and minimal side effects [8,9].

Carbamazepine, 5-H-dibenze[b,f]azepine-5-carboxamide (Fig. 1), is a tricyclic lipophilic compound that is a first-line antiepileptic drug [10]. It particularly shows good antiepileptic activity by blocking sodium channels [11].
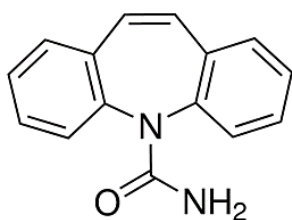


**Fig. 1.Carbamazepine's chemical structure**

Various analytical methods have been published in the literature for the determination of carbamazepine in pharmaceutical preparations, including spectrophotometry [12], FT-Raman spectroscopy[13], micellar electrokinetic capillary chromatography, spectrofluorimetry [14], fluorescence polarization immunoassay [15], chemiluminescence [16] and gas chromatography-mass spectrometry [17]. Many of these analytical methods are complex and time consuming, requiring expensive instruments and specialized sample preparation techniques. Analytical methods for HPLC determination of Carbamazepine and its impurities have been reported in European Pharmacopoeia, United States Pharmacopoeia, British Pharmacopoeia and Indian Pharmacopoeia. Some pretreatment techniques such asliquid-liquid extraction [18, 19], solid-phase extraction [20-23], solid phase micro extraction [24], stir bar-sorptive extraction [25] ,and deproteinization [26] have been used for thedetermination of Carbamazepine and its metabolites by HPLC in plasma.This is why HPLC has come to the fore as a reliable technique for the quantification of these and other anticonvulsant drugs [27- 34].

In all these studies, there are no studies in which two different analysis methods have been developed and the methods have been compared statistically. Therefore, the purpose of this investigation was to develop and validate a chromatographic and spectrophotometric methods that could be used for determination of Carbamazepine in pure forms and in pharmaceutical formulations. The results of these methods have been compared statistically using variance analysis. We also evaluated the effectiveness and applicability of the methods, focusing on quality control research.

## 2. MATERIALS AND METHODS

### 2.1 Materials

All chemical compounds were analytical and HPLC grade. Potassium dihydrogen phosphate (99.9%), methanol (≥ 99.9%), and acetonitrile (≥ 99.9%), were bouht from Sigma-Aldrich, Turkey.Carbamazepine reference standard and

tablets containing 200 mg of carbamazepine per tablet (Tegretol) was obtained from Novartis,Turkey. The ultra pure water used in this study was produced using a Milli-Q water purification system(Millipore, USA).

## 2.2 Conditions of Chromatographic and Spectrophotometric Methods

Spectrophotometric analysis were performed on the Shimadzu 1800 dual-beam UV-Vis spectrophotometer, consisting of UV-Probe software and 1.0 cm quartz cells. For carbamazepine quantification, a wavelength of 286 nm was chosen and methanol was used as a blank for the absorbance measurements.

The chromatographic analysis were performedusing an Agilent 1260 LC system composed of UV detector and Chemstationsoftware. An Extend-C18 column (250 mm × 4.6 mm, 5µm particle diameter) was used and the mobile phase consisted of 20 mM potassium dihydrogen phosphate solution(pH adjusted to 3.5 with orthophosphoric acid)and acetonitrile (40:60) at a flow rate of 1.2 mlmin$^{-1}$. Detection was carried out on at 286 nm.

## 2.3 Standard and Sample Solutions of Carbamazepine

The stock standard solution was prepared by dissolving carbamazepine with methanol to obtain concentration of 500 µg mL$^{-1}$. Standard solutions of carbamazepine in the concentration range of 5-25 µg mL$^{-1}$ were prepared by diluting the stock standard solution with methanol for both methods. The absorbance of standard solutions was measured. It was found that the absorbance of standard solutions was proportional to the corresponding concentrations of carbamazepine.Twenty microlitres of each standard solution were injected into the HPLC system. The calibration graph was created by plotting the peak areas against the matching concentrations.

A total of 10 tablets containing 200 mg of carbamazepine as the active ingredients were weighed and finely powdered. The powder equivalent to 50 mg of carbamazepine was taken in 100 mL of volumetric flask and dissolved in methanol. The contents of the flask were sonicated for 10 minutes to dissolve carbamazepine thoroughly, the final mixture was filtered. From this stock sample solution, test solutions were prepared in a concentration of 20 µg mL$^{-1}$.

## 2.4 Determination of λ$_{max}$

First, the spectrophotometer was calibrated to zero. Then the maximum absorption wavelength of carbamazepine solution (20 µg mL$^{-1}$) was determined by scanning in the range of 200 and 800 nm.

## 2.5 Method Validation

The optimized analytical methods have been validated in compliance with the recommendations of the ICH guidelines [35,36]. Validation parameters (System suiyability, linearity, selectivity, accuracy, precision, robustness, and limit of detection and quantification) have been investigated.

System suitability test was performed with respect to injection repeatability (R.S.D. of retention time and peak area response), tailing factor, peak symmetry, and theoretical plate number using a standard solution (Carbamazepine, 20 µg mL$^{-1}$) [37-42].

Linearity was investigated by examining five standard solutions (n = 3) ranging from 5 to 25 µg mL$^{-1}$ for both methods. The stock standard solution (500 µg mL$^{-1}$) containing of carbamazepine in methanol were prepared in triplicate.These aliquots solutions were diluted to five different concentrations, corresponding to of 5-25 of carbamazepine for both methods. Calibration curves with concentration values against absorbance or peak area were drawn for each methodand the data from both analytical methods were regression analyzed using the least-squares method.

Selectivity of both methods were assessed by comparison of the spectrums and chromatograms obtained from standard and sample preparations which take part in the pharmaceutical preparations. To assess any interfering peaks, a sample solution was prepared and injected into the chromatograph. For spectrophotometric analysis, the UV spectrum of the sample solution was recorded in the range of 200-800 nm to evaluate the presence of possible interfering bands at 286 nm.

Precision of both methods were analyzed in terms of both repeatability (intraday precision) and intermediate precision (interday precision).Repeatability was performed by analyzing the sample solution six times in the

same day for both methods. Similarly, intraday and interday precision was assessed by analyzing the sample solution on the same and different days, respectively. Carbamazepine contents and the relative standard deviation (R.S.D.) values were calculated.

Analytical recovery tests were carried out using the standard addition method to ensure that the proposed procedures were accurate and to investigate the effects of formulation additives. The sample solutions were prepared in triplicate et each level and carbamazepine standard solutionwas added to the sample solutions. Average recovery percentages and R.S.D.% values were determined for both methods.

The limits of detection (LOD) and quantitaton (LOQ) were calculated independently using equations (1) and (2), depending on the standard deviation of the y-intercept and the slope of the calibration curve, respectively.

$$LOD = 3.3 * δ/S \quad (1)$$
$$LOQ = 10 * δ /S \quad (2)$$

Where, S: slope of calibration curve and δ: standard deviation of y-intercept. A standard solution at LOQ concentration was prepared. For precision and accuracy tests, This solution was analyzed 6 replicates on the same day. The precision test result was determined as R.S.D%. Accuracy test results were calculated as recovery %.

The robustness of analytical methods was evaluated by making small changes in method conditions. For HPLC method, samples have been analyzed under different circumstances like changes in the flow rate of mobile phase (±0.1 mL min$^{-1}$) and in acetonitrile content (±2 %) in the mobile phase and the effect of system suitability parameters have been observed. For the UV method, samples have been analyzed under different conditions such as using different brands of methanol (Sigma-Aldrich, 34860/J.T. Baker 8402) as solvent and detection wavelengths (±2 nm).

## 2.6 Statistical Comparison of Methods

From the validation results, it was determined that the above-mentioned methods were suitable for routine quality control analysis of Carbamazepine in commercial formulations. The recovery percentages were statistically compared when both methods were applied to a commercial drug formulation. For this purpose, F-test and t-test and were applied.

## 3. RESULTS AND DISCUSSION

### 3.1 Method Development

Because methanol completely and easily dissolves carbamazepine, methanol was chosen as the solvent to obtained UV spectrum in the range of 200-800 nm [Fig. 3]. The wavelength of 286 nm was selected for measurement, due to the adequate molar absorptivity of carbamazepine in this region after the evaluation of the spectrum,

The chromatographic method was optimized by changing the column, flow rate, and mobile phase composition.Finally development was carried out using Extend- $C_{18}$ (250 mm × 4.6 mm i.d., 5 μm particle size) column and a mobile phase composed of acetonitrile and 20 mM potassium dihydrogen phosphate solution (pH adjusted to 3.5 with orthophosphoric acid) (60:40 v/v). The eluent was observed at 286 nm. Fig. 2 displays chromatogram produced of the carbamazepine standard and sample solutions using developed method. As indicated in the chromatogram, acceptable peak symmetry and a short run time were attainedas seen in this figure. Table 1 illustrates the system suitability parameters.

Selectivity of the LC method was assessed by checking that no interference peaks were found at the retention times of Carbamazepine with mobile phase blank and tablet sample solutions. For this, chromatograms of solutions of standard (25 μg mL$^{-1}$), tablet sample (20 μg mL$^{-1}$), and mobile phase blanks were compared. The chromatograms of standard and tablet samples showed peaks for Carbamazepine without any interfering peaks. In mobile phase blank chromatogram, no peak was observed at the retention time of Carbamazepine in Fig. 2. Thus the method was proved selective. The selectivity of the UV method, the spectra of the standard, blank and tablet sample solutions were compared, and no interference was observed. Thus the method was selectively proved [Fig. 3].

### 3.2 Linearity

It was discovered that the concentration of standard solutions and the response had a linear relationship in both methods. The data of

regression analysis were illustrated in Table 2. The resulting regression coefficients ($r^2$) were higher than 0.999, which proved the linearity of these methods.

**Table 1. System suitability data (n=5)**

| Parameters | Carbamazepine |
|---|---|
| Retention time of carbamazepine peak (min) | 5.18 |
| Peak tailing factor | 1.22 |
| Theoretical plates (N) | 6353 |
| Capacity factor | 1.35 |



A



B



C



D

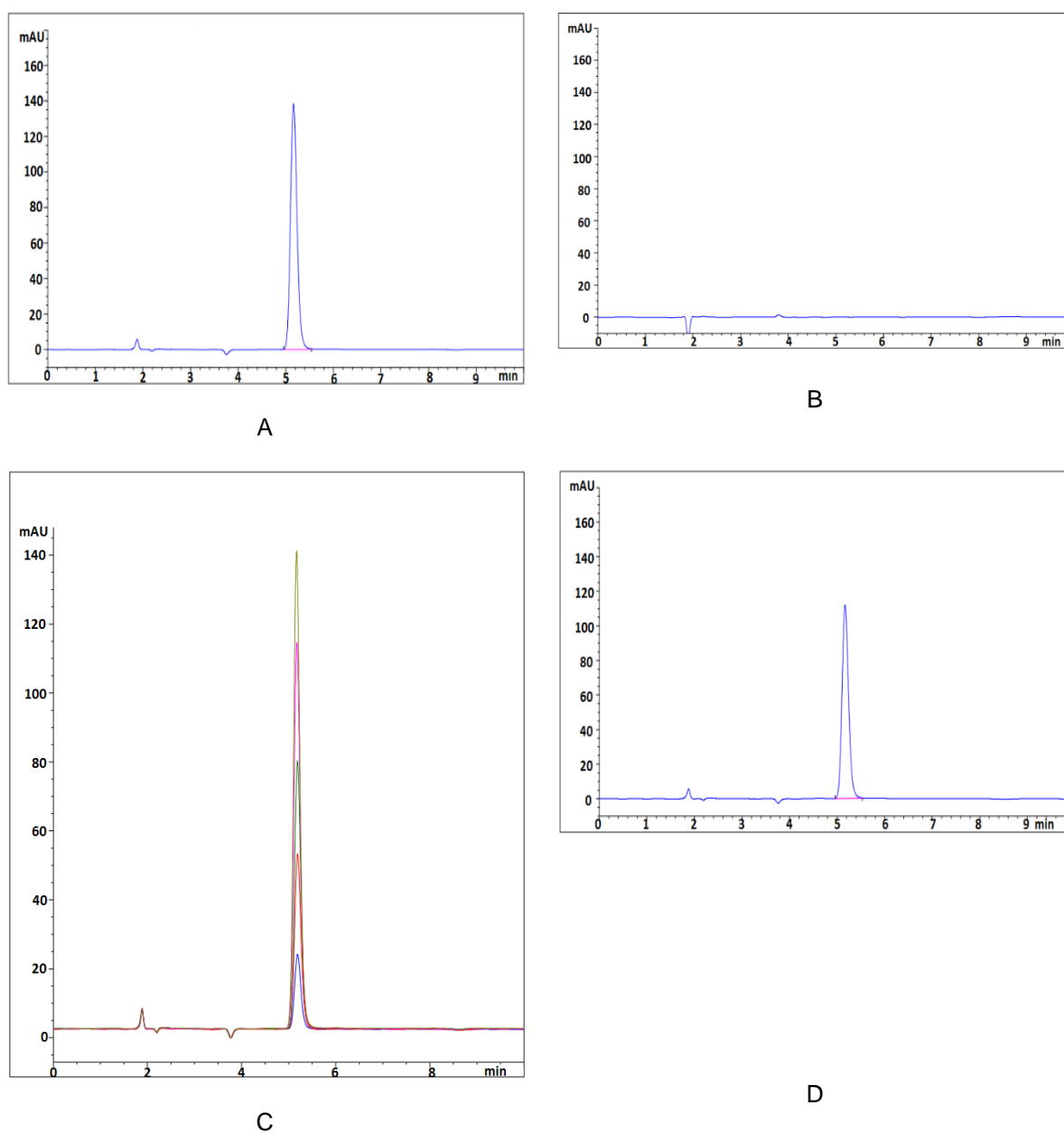**Fig. 2. A. Chromatogram of standard carbamazepine solution (25 μg mL⁻¹),B. Chromatogram of blank solution, C. Overlap chromatogram of standard solutions (5-25 μg mL⁻¹), D. Chromatogram of sample solution (20 μg mL⁻¹)**
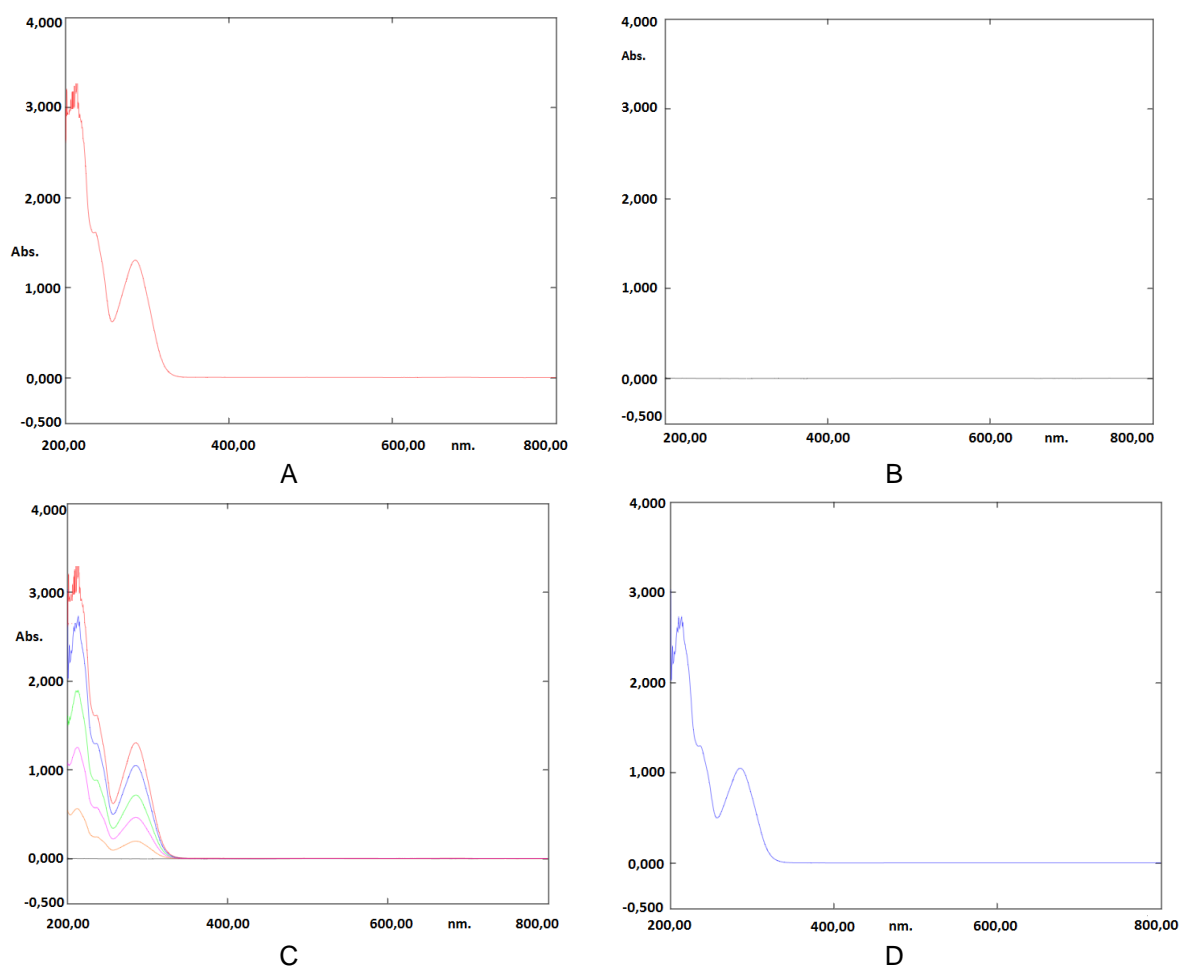
**Fig. 3. A. Spectrum of standard carbamazepine solution (25 µg mL$^{-1}$), B. Spectrum of blank solution, C. Overlap spectrum of standard solutions (5-25 µg mL$^{-1}$), D. Spectrum of sample solution (20 µg mL$^{-1}$)**

**Table 2. Linearity data of methods**

| Regression parameters | UV | LC |
|---|---|---|
| Concentration range (µg mL$^{-1}$) | 5-25 | 5-25 |
| Number of points | 5 | 5 |
| Correlation cefficient (r$^2$) | 0.9995 | 0.9999 |
| Slope | 0.0555 | 56.521 |
| Intercept | -0.015 | -78.208 |
| Calibration curve | y = 0.0555 x - 0.015 | y = 56.521 x - 78.208 |
| Precision (RSD %, *n*=6) | 0.36 | 0.22 |
| Recovery (RSD %, *n*=9) | 1.17 | 0.80 |
| LOD/LOQ (µg mL$^{-1}$) | 1.6/4,7 | 0.7/2,2 |
| Intraday | 0.2576 | 0.1569 |
| Interday | 0.4971 | 0.4001 |

Limit of detection and quantification have been found to be 0.70 and 2.20 µg mL$^{-1}$ for chromatographic method, 1.60 and 4.70 µg mL$^{-1}$ for spectrophotometric method respectively (Table 2). Standard solutions were prepared at LOQ concentrations (2.20 µg mL$^{-1}$ for LC, 4.70 µg mL$^{-1}$ for UV). For precision and accuracy tests, 6 replicates were analyzed at LOQ concentration on the same day. As a result of the precision test, R.S.D was determined as 1.50%. The accuracy test results at LOQ concentration were between 98.85-101.10%.

### 3.3 Precision

R.S.D.% values of both analytical methods are lower than 2. This indicates good precision; however, the chromatographic method is more precise than the spectrophotometric method.

### 3.4 Accuracy

Accuracy was assessed by recovery studies utilizing both methods. The mean recoveries (*n* = 9) for both spectrophotometric and chromatographic procedures were close to 100 percent [Tables 3], indicating appropriate accuracy.

### 3.5 Robustness

The results of robustness were presented in Table 4.No significant changes in the system suitability parameters were observed when the organic content and flow rate of the mobile phase

were changed. The low R. S. D.% values showed that the method was sufficiently robust.

### 3.6 Specificity

The chromatogram obtained from sample solution containing excipients did not show any interfering peaks in the retention time of carbamazepine for HPLC method.There was no interfering absorption band at 286 nm in the spectra of the sample solution for UV method.

### 3.7 Analysis of Pharmaceutical Formulations

The quantitative results using UV and LC methods were given in Table 5. Although, when compared to LC method, UV method had a slightly higher R.S.D. % value. the difference in mean values was not statistically significant.As both analytical methods have been determined to be accurate and precise, they can be used for routine quality control analysis of carbamazepine

### Table 3. Recovery data

| Analytical method | Standard addition level % | Amount spiked ($\mu g\ mL^{-1}$) | Amount recovered ($\mu g\ mL^{-1}$) | Mean recovery % | R.S.D. % (*n*=3) |
|---|---|---|---|---|---|
| UV method | 80 | 8 | 8.02 | 100.09±0.1723 | 0.1721 |
| | 100 | 10 | 9.96 | 99.84±0.2022 | 0,2025 |
| | 120 | 12 | 12.06 | 100.22±0.2107 | 0,2102 |
| LC method | 80 | 8 | 7.97 | 99.86±0.091 | 0.0913 |
| | 100 | 10 | 10.03 | 100.12±0.1395 | 0,1393 |
| | 120 | 12 | 11.98 | 99.93±0.0011 | 0,1051 |

### Table 4. Robustness data

| Method | Parameter | Value | Tailing factor | Number of theoretical plates | Content % |
|---|---|---|---|---|---|
| **LC method** | Acetonitrile composition (%) | 58 | 0.875 | 6324 | 100.07 |
| | | 62 | 0.839 | 6343 | 99.92 |
| | Flow rate ($mL\ min^{-1}$) | 1.1 | 0.853 | 6352 | 99.87 |
| | | 1.3 | 0.846 | 6337 | 100.02 |
| **UV method** | Solvent | Methanol Sigma-Aldrich Cat: 34860 | | | 100.07 |
| | | Methanol JT Baker Cat: 8402.2500 | | | 99.90 |
| | Detection wavelengths | 284 | | | 99.89 |
| | | 288 | | | 100.08 |

**Table 5. Statistical comparison of analytical techniques**

| Drug | Label claim mg per tablet | Parameter (n=6) | UV method | LC method |
|---|---|---|---|---|
| Tegretol | 200 | Mean % | 99.84 | 99.92 |
| | | Amount found (mg) | 199.25 | 199.64 |
| | | S.D.* | 0.2205 | 0.1854 |
| | | R.S.D. %* | 0.2209 | 0.1855 |

*\* S.D.: Standard Deviation; R.S.D.: Relative Standard Deviation*

**Table 6. Statistical comparison results of LC and UV methods (α=0.05, 95% confidence interval, n=6)**

| Statistical values | LC Method | UV Method |
|---|---|---|
| Average value | 100.25 | 100.55 |
| Standard deviation (S.D.) | 0.75 | 1.35 |
| Relative standard deviation (R.S.D.%) | 0.75 | 1.34 |
| Standard error | 0.34 | 0.87 |
| F-testi | 0.27/0.44 | |
| $F_{calculation}/F_{table}$ | | |
| t-testi | 1.75/2.82 | |
| $t_{calculation}/t_{table}$ | | |

## 3.8 Statistical Comparison of Methods

F-test and t-test and were applied for statistical comparison of both methods. Statistical analyzes revealed that there was no significant difference between the values obtained from the analyzes performed by both methods. The calculated t-value and F-value were found to be lower than the table values of both methods in the 95% confidence interval. It is clear from this report that both of the recommended UV and LC methods are applicable to the determination of Carbamazepine in drug formulations appropriately. Statistical comparison results of LC and UV methods has been shown in Table 6.

## 4. DISCUSSION

In this study, two different methods, High Performance Liquid Chromatography, which are frequently used in drug analysis, and spectrophotometric method, were developed in order to determine the amount of Carbamazepine active ingredient in pharmaceutical formulations. At the same time, the chromatographic and spectrophotometric conditions of these developed methods were optimized.

For quantification of Carbamazepine in pharmaceutical formulations, a number of analytical procedures have been published. Some of these methods are complex. These methods require expensive instruments, a large amounts of organic solvents and special reagents. Analysis times are long. In addition, the spectrophotometric and spectrofluorimetric methods presented in the literature involve complex and long sample preparation steps.In all these studies, there is not yet a study in which two different analysis methods were developed and the methods were compared statistically.

## 5. CONCLUSIONS

The suggested UV method has an advantage over LC method in that it does not necessitate the complicated procedures and treatment associated with chromatographic methods.It takes less time and is more cost-effective.LC method is more accurate and precise than UV method in terms of quantitative determination of carbamazepine, according to a statistical comparison.The results show that LC and UV spectroscopy methods are suitable techniques for quantifying carbamazepine in pure form and dose form.Excipients in the tablets had no effect on the results and it is simple to prepare the mobile phase.Since these methods are rapid, specific, precise, accurate, and simple, they can be used for quality control analysis of carbamazepine in pharmaceutical formulations, and bulk.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our

area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

İt is not applicable.

## ETHICAL APPROVAL

we conducted our research after obtaining proper IEC approval.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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