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DETERMINATION OF CARBAMAZEPINE IN PHARMACEUTICAL PREPARATIONS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND DERIVATIVE SPECTROPHOTOMETRY

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Abstract

A sensitive reversed phase high-performance liquid chromatographic (HPLC) and second derivative spectrophotometric methods for determination of carbamazepine in tablets have been developed. In the HPLC method, carbamazepine was separated using Phenomenex C18 column and acetonitrile:water (75:25,v/v) the mobile phase system; the speed of the mobile phase flowing was 1mL/min and the detection was actualized at 285 nm. Enalapril was used as an internal standard. For the second-derivative spectrophotometric method, carbamazepine was determined by applying the technique of the "peak to peak amplitudes".The assay was linear over the concentration range of 0.2-2.0 μg/mL for HPLC and 4.0-10.0 μg/mL for second derivative spectrophotometric method. The detection limits of carbamazepine were 0.055 and 1.25 μg/mL for HPLC and derivative spectrophotometric methods, respectively. The recovery (mean±RSD) in HPLC was 99.22±0.25% and in the derivative spectrophotometry 99.05±0.25%. The proposed methods were successfully applied to the determination of carbamazepine in tablets: the recovery was of high percentage, the accuracy and presicion were good.

Key words:*Carbamazepine, Derivative spectrophotometry, High-performance liquid chromatography,Validation*

Karbamazepinin Yüksek Performanslı Sıvı Kromatografi ve Türev Spektrofotometri ile Farmasötik Preparatlarda Miktar Tayini

Karbamazepinin tabletlerde miktar tayini için ters faz yüksek performanslı sıvı kromatografisi ve türev spektrofotometrisi yöntemleri geliştirildi. HPLC yönteminde karbamazepin phenomenex C18 kolon ve acetonitrile:su (75:25,v/v) mobil faz sistemi kullanılarak ayrıldı, mobil faz akış hızı 1 mL/min ve teşhis 285 nm de yapıldı. Enalapril internal standard olarak kullanıldı. 2. türev spektrofotometrik yöntem için karbamazepin pikten pike ölçme tekniği uygulanarak tayin edildi. Lineer konsantrasyon aralığı HPLC yöntem için 0.2-2.0 μg/mL ve 2.türev spektrofotometrik yöntem için 4.0-10.0 μg/mL dır. Carbamazepinin teşhis sınırları HPLC yöntem için 0.055 ve 2.türev spektrofotometrik yöntem için 1.25 μg/mL dır. Geri kazanım (ortalama ± bağıl standart sapma) HPLC de % 99.22±0.25% ve 2.türev spektrofotometrik yöntemde ise 99.05 ± 0.25% dır. Geliştirilen yöntemler karbamazepin içeren tabletlerde miktar tayinine güvenle uygulandı, geri kazanım yüksek doğruluğu ve kesinliği iyidir.

Anahtar Kelimeler: *Karbamazepin, Türev spektrofotometrisi, Yüksek performanslı sıvı kromatografisi, Validasyon*

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INTRODUCTION

Carbamazepine (CAR) is an anticonvulsant and specific analgesic for trigeminal neuralgia. Its chemical name is 5H-dibenz (b,f)azepine-5-carboxamide, and its structural formula is shown in (1).

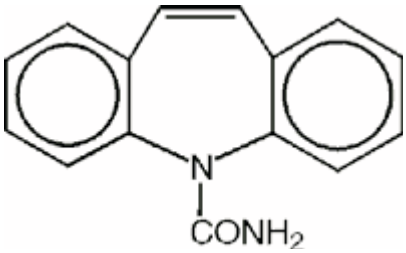

Figure 1. Chemical structure of Carbamazepine

Several methods have been presented in the literature for the determination of CAR in pharmaceutical preparations and biological fluids.

Determination of CAR was made in pharmaceutical preparations by electrolysis-fluorimetric (2) and near infrared FT-Raman spectroscopy methods (3). Zhang et al. (4) have proposed a method for the determination of CAR by polarography in tablets. Huang et al. (5) have developed flow injection photochemical spectrofluorimetry for the determination of CAR in pharmaceutical preparations. Abdel-Hamid (6) has studied comparative LC–MS and HPLC analyses of selected antiepileptics and beta-blocking drugs. Hanada et al. (7) have reported stability-indicating HPLC method for CAR.

Various analytical techniques have been employed for the determination of CAR in biological fluids. Determination of CAR was studied by HPLC (8-23), tandem liquid chromatography–mass spectrometry with electrospray ionization (24-26) and by miceller electrokinetic capillary chromatographic (27) methods in human plasma.

In the present work, we developed rapid, accurate and sensitive HPLC and derivative spectrophotometric methods for the determination of CAR in tablet dosage form. For more specificity, a simple isocratic high performance liquid chromatographic method was developed. The two proposed methods are fairly accurate, rapid, reproducible, sensitive and have wide linear range. The derivative spectrophotometric method has the advantages of the speed and low cost.

EXPERIMENTAL

Materials

CAR and Enalapril (Internal standard, IS) were kindly supplied from Eczacıbaşı (Istanbul, Turkey) and Deva (Istanbul, Turkey), respectively. Tegretol® 200 mg was supplied from Novartis (Istanbul, Turkey). HPLC grade acetonitrile and methanol solvents were purchased from Merck (Darmstadt, Germany). HPLC grade water was provided by aquaMAXTM (Young Instrument, Korea). All solvents used were analytical grade.

Apparatus

A Thermo Separation Products (Texas, USA) Liquid Chromatograph, consisting of a Model P solvent delivery system, a Rheodyne injection system with a loop of 20 µL was used. The mobile phase was prepared by mixing acetonitrile and water in the ratio of 75:25. All the determinations were performed at ambient temperature (20°C) using Phenomenex-C18 Column (Aqua, 150 mm × 4.6 mm i.d. with 5 µm particle size, Thermo Separation, TX, USA), with a guard column (4 mm x 3 mm ID, Phenomenex) packed with the same material. The column effluent was monitored at 285 nm. Chromatograms were recorded by means of a computer and were treated as software SN 4000. The injection volume was 20 µL with a flow rate of 1 mL/min. A Shimadzu UV-160A UV-Visible spectrophotometer with 1 cm quartz cells was used under the following operating conditions: Wavelength scanning speed, 2400 nm/min, scan range 200-400 nm, slit width 2 nm and derivation interval ($\Delta\lambda$) 2.8 nm.

Solutions

HPLC method

Stock solutions of CAR and IS (1.0 mg/mL) were prepared with methanol and these solutions were diluted with methanol to give working solutions at 100 µg/mL concentrations. Standard solutions of CAR in the concentration range of 0.2-2.0 µg/mL containing at a fixed concentration (1.0 µg/mL) of IS were prepared in the mobile phase. Triplicate 20 µL injections were made for each concentration and the ratio of the peak area of CAR in each concentration to the peak area of internal standard was plotted against the corresponding concentrations to obtain the calibration graph.

Derivative method

A stock solution was prepared by dissolving CAR in methanol to obtain a concentration 100 µg/mL. The standard solutions were prepared by dilution of the stock solution with methanol to

reach concentration ranges of 4.0–10.0 µg/mL. The distance between two extremum values (peak-to-peak amplitudes), 257.5/286.0 were measured in the second derivative (^{2}D) spectra of standard solutions. These wavelengths were selected depending on the maximum values obtained (Figure 2). Calibration curves were constructed by plotting ^{2}D values against concentrations.

Method validation

The validation of RP-HPLC method developed for the determination of CAR was carried out under the guidelines of the International Conference Harmonization (ICH) (28, 29). Calibration curves were obtained with five concentrations of the standard solutions (n = 6). In HPLC method, the CAR/IS peak area ratios were plotted as a function of CAR concentrations. In derivative spectrophotometry, the calibration curves were set up by plotting ^{2}D values for the respective drug concentrations. The limit of detection (LOD) and the limit of quantification (LOQ) may also be calculated based on the Standard Deviation (SD) of the response and the slope (S) of the calibration curve(s) at levels approximating the LOD according to the formula: LOD=3.3(SD/S) and LOQ=10(SD/S). The SD of the response can be determined based on the SD of the blank, on the residual SD of the regression line, or the SD of y-intercepts of regression lines, under the ICH guidelines (28,29). The accuracy and precision were assessed by determining CAR samples at three concentration levels on 3 different days. The recoveries of the proposed methods were tested with the standard addition technique by adding a known amount of standard at three different levels to the pre-analyzed sample. The specificity of the two methods was investigated by observing interferences between CAR and the excipients (2.0 µg/mL for HPLC and 10 µg/mL for derivative spectrophotometric). The robustness of the proposed methods was determined by analysis of samples under a variety of conditions such as small changes in wavelength (nm), flow rate (mL min^{-1}) and mobile phase composition. To ascertain the resolution and reproducibility of the HPLC method, system suitability tests were carried out using working standard solution of CAR. Resolution (Rs), N (theoretical plates), k' (capacity factor) and T (tailing factor) were measured as the criteria for system suitability testing.

Assay procedure for tablets

Twenty tablets were weighted and powdered, respectively. An appropriate amount of this powder, equivalent to 10 mg of CAR was placed in with 100 mL volumetric flask with and suspended in 50 ml of methanol. The solution was sonicated for 20 min and diluted to volume with the same solvent. Then the solution was filtered. The filtrate was diluted to obtain the appropriate concentrations (similar to standard working solutions,100 µg/mL).

For HPLC analysis, 0.8 mL aliquots of the resulting solution were transferred into a 100 mL

volumetric flask, 1.0 mL of the internal standard solution was added and the volume was adjusted to volume with the mobile phase. A volume of 20 µL was injected into the chromatograph concurrently with the appropriate standard solution (0.8 µg/mL) and the peak area ratios (drug to internal standard) were used for the determination of CAR in each sample. For derivative spectrophotometric method, a 0.8 mL aliquot of the resulting solution was transferred to a 10 mL volumetric flask and the volume was adjusted with methanol. The ^{2}D amplitudes were measured directly as described under the calibration graph and the concentration of CAR in each sample was determined in the appropriate calibration graph.

RESULTS AND DISCUSSION

After choosing the C18 column as optimal, the influence of other chromatographic conditions on separation was investigated. As mobile phase, different mixtures of water and acetonitrile (starting from ratio 80:20, v/v, to ratio 60:40, v/v) were tested. Then usage of phosphate buffers (0.05 M KH_2PO_4 adjusted to pH 4.0 with phosphoric acid and 0.05 M KH_2PO_4 adjusted to pH 6.0 with 1 M potassium hydroxide) instead of water and subsequent influence on separation were investigated. The developed HPLC method based on using a reversed phase, C18 column, with a mobile phase consisting of acetonitrile–water in a ratio 75:25 v/v with flow rate of 1 ml per min at ambient temperature, yielded the best results. In this condition, CAR eluted at 2.37 min and IS at 1.14 (Figure 3). Besides, according to the other methods the retention time is quite short (8-12,18,21,24). On the other hand, the mobile phase system used was quite simple and there was no need for a buffer system and washing the column (8-12,18,21,24). In the HPLC method calibration curves were constructed using peak area ratios of CAR to the IS and CAR concentrations. Linear regression analysis was used to calculate the slope, intercept, and correlation coefficient (r^2). The linearity was over the range of 0.2-2.0 µg/mL, which are as good or superior to that reported in the other papers (21). The limit of detection (LOD) represents the concentration of CAR that would yield a signal-to-noise ratio of 3 in accordance with ICH guidelines (28,29). LOD and LOQ were 0.055 and 0.167 µg/mL, respectively.

In this study to obtain more sensitive results; the first, second, third and fourth derivative spectra of CAR was achieved and then it was determined that second derivative was the best. Calibration curves were constructed by plotting d2A/dλ2 values against concentrations of CAR. Linear relationships were observed over the concentration ranges of 4.0-10.0 g/mL. LOD and LOQ were found to be 1.25 and 3.80 µg/mL, respectively (Table 1). Calibration curves of CAR were linear over the concentration range of 0.2-2.0 µg/mL for HPLC method and 4.0-10.0 µg/mL for derivative spectrophotometric method, which is as good as or superior to that reported in other papers (4,6,10-12,21,24).

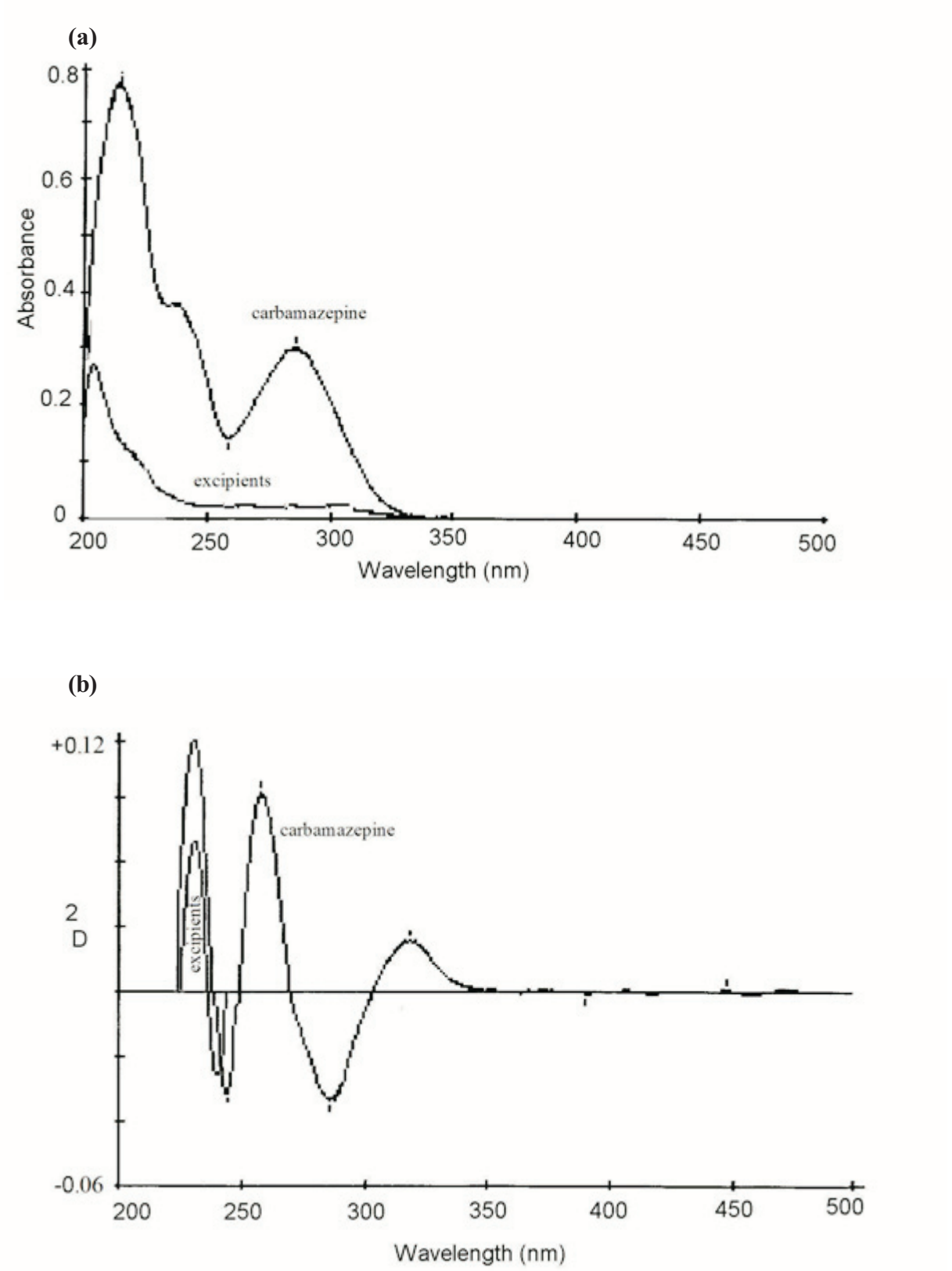

Figure 2. Zero-order UV spectra of 4.0 µg/mL carbamazepine and 10 µg/mL excipients inmethanol (a), Second - order derivative spectra of 4.0 µg/mL carbamazepine and 10 µg/mL excipients in methanol (b)

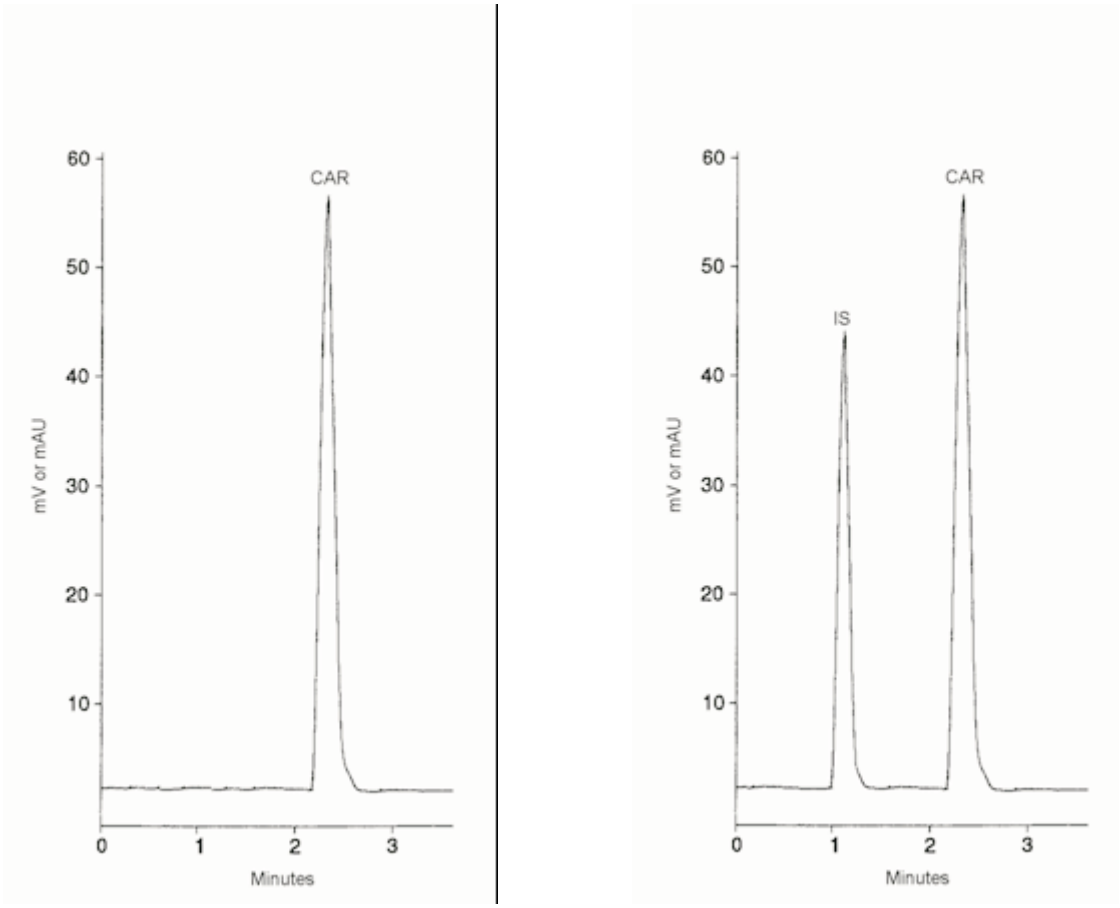

Figure 3. Chromatogram of carbamazepine (2.0 µg/mL) without IS (a) and with IS (b).Mobile phase, acetonitrile-water (75:25 v:v), flow rate 1 mL/min and detection wavelength 285 nm.

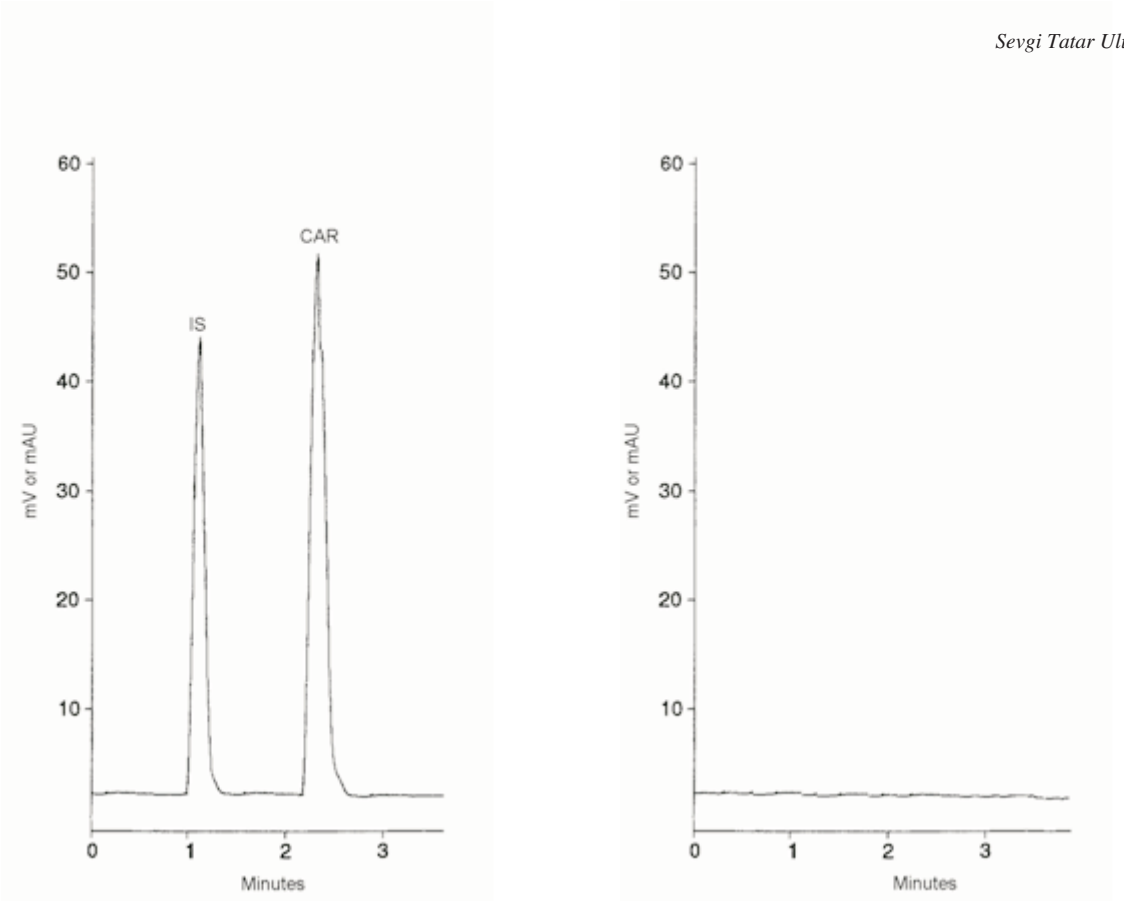

Figure 4. Chromatogram of carbamazepine tablet solution (2.0 µg/mL) and IS (1.0 µg/mL) (a), an d excipients (2.0 µg/mL) (b). Mobile phase, acetonitrile – water (75:25 v:v), flow rate 1 mL/min and detection wavelength 285 nm.

Table 1. Statistical analysis for calibration curves of CAR by HPLC and derivative spectrophotometric methods

	HPC	Derivative spectrophotometry
Analytical wavelength (nm)	285	270.6/282.6
Linearity range (µg/mL)	0.2-2.0	4.0-10.0
Regression equation	$A = 0.264C + 0.023$	$D = 0.725C - 1.5213$
SD of slope ($n = 6$)	5.3x10	2.1x10
SD of intercept ($n = 6$)	4.4x10	0.2755
LOD=3.3(SD/Slope)	0.055	1.25
LOQ=10(SD/Slope)	0.167	3.80
Correlation coefficient	0.9998	0.9996

Table 2. Precision and accuracy of HPLC and derivative spectrophotometric methods (n=6)

Method	Drug concentration (µg/mL)	%RSD	%RME
Intra day			
HPLC			
	0.2	1.21	-3.50
	0.4	0.87	-2.60
	0.6	0.45	-1.20
	1.0	0.23	-0.78
Derivative	4.0	0.38	-2.85
Spectrophotometry	6.0	0.26	-2.24
	8.0	0.19	-1.88
	10.0	0.12	-1.45
Inter day			
HPLC			
	0.2	1.27	-3.80
	0.4	0.91	-2.78
	0.6	0.49	-1.35
	1.0	0.27	-0.98
Derivative	4.0	0.42	-3.15
Spectrophotometry	6.0	0.33	-2.76
	8.0	0.23	-2.12
	10.0	0.16	-1.67

RSD (Relative Standard Deviation)

RME (Relative Mean Error)

Table 3. Recovery of the proposed methods (standard addition method, n=7)

HPLC method *Tablet solution*	*Standard solution (µg/mL)*	Recovery(%) (mean ± SD)	RSD (%)
0.4	0.2	$98.89 \pm 3.1x10^{-1}$	0. 31
0.4	0.4	$99.21 \pm 2.6x10^{-1}$	0. 26
0.4	0.6	$99.56 \pm 1.8x10^{-1}$	0. 18
Derivative spectrophotometry *Tablet solution*	*Standard solution (µg/mL)*	Recovery(%) (mean ± SD)	RSD (%)
4.0	2.0	$98.56 \pm 4.2x10^{-1}$	0. 42
4.0	4.0	$99.12 \pm 3.5x10^{-1}$	0. 35
4.0	6.0	$99.46 \pm 2.8x10^{-1}$	0. 28

Table 4. Experimental data for robustness testing and obtained responses

Parameter	Modification	CAR (% recovery)
Mobile phase ratio (v/v) acetonitrile water	75:25 ***(Optimum)***	99.12
	70:30	98.97
	80:20	98.89
Flow rate (mL/min)	1.0 ***(Optimum)***	99.12
	1.1	98.85
	0.9	98.91
Wavelength (λmax, nm)	285 nm ***(Optimum)***	99.12
	284 nm,	98.43
	286 nm,	98.38
	λ:270.6/282.6***(Optimum)***	98.92
	λ: 271/283 nm	98.76
	λ: 269/281 nm	98.68

Table 5. System suitability data of proposed HPLC method

Parameters	CAR
Retention time (min)	2.37
Capacity factor	3.74
Tailing factor	0.72
Therotical plates/meter	7105
HETP (mm)	0.141

Table 6. Comparison of the results obtained by proposed methods and official method (*n*=6) Tegretol ® tablet (200 mg)

Statistical value	HPLC method	Derivative spectrophotometric method	Official method [30]
Mean ± SAE	198.97± 0.095	198.52 ± 0.135	198.45 ± 0.145
Recovery (%)	99.49	99.26	99.23
RSD (%)	0.43	0.53	0.57
t-test of significance	0.91	0.78	
F-test of significance	1.73	1.98	

n=6, p=0.05, t=2.23, F=5.05, SAE, Standard Analytical Error

The evaluation of method precision was carried out in a day (intraday precision) and in three different days (interday precision) and evaluated by means of the RSD. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte. It was tested by using six replicates for each concentration level of CAR intra- and interday (in three different days). To evaluate this parameter relative mean error (RME) was used. The things that can be seen in Table 2, the obtained values for precision (RSD < 0.91 % for HPLC and RSD < 0.42 % for derivative method) and accuracy (RME < 3.80% for HPLC and RME < 3.15% for derivative method). As shown in Table 2 the repeatability and reproducibility of two methods are fairly good as indicated by the low values of RSD%.

The recovery study was performed by adding known amounts of the compounds studied to a known concentration of the commercial pharmaceutical tablets (standard addition method).The resulting mixtures were analyzed and results obtained were compared with the expected results. The mean recoveries for CAR at 0.6, 0.8 and 1.0 (total concentration) were 98.89± 0. 31%, 99.21 ± 0. 26 and 99.56 ± 0. 18 (n = 7), respectively. The mean relative recovery for IS at 1.0 µg/mL was 98.12 ± 1.12% (n = 6). For derivative spectrophotometric method, the mean recoveries for CAR at 6.0, 8.0 and 10.0 µg/mL were 98.56 ± 0.42%, 99.12 ± 0.35 and 99.46 ± 0.28 (n = 7), respectively (Table 3). The recovery is quite high if we consider the one method in the literature (6).

The excipients chosen are the ones commonly used in tablet formulation, which included mannitol, starch, sucrose, magnesium stearate and stearic acid . The specificity of two methods was proved by observing no interference encountered from the excipients of the tablets (Figure 2 and 4).

The result of the robustness of the assay method is demonstrated in Table 4. Method robustness checked after deliberate alterations of mobile phase composition, flow rate and wavelength shows that the changes of the operational parameters do not lead to essential changes of the performance of the chromatographic system. The proposed methods conditions are robust. It was concluded that the developed method is the optimum according to the studied parameters.

The capacity factor obtained is within the accepted values, 1<k'<10. The values of the number of theoretical plates were higher than the accepted value of 2000 and tailing factor T < 2 (minimum value to consider, it is an acceptable method) (28,29). Therefore, HPLC method can be applied to routine analysis with no problems, its suitability being proved (Table 5).

Application to pharmaceutical preparations

Commercially available tablets of CAR (Tegretol® 200 mg) were subjected to analysis by

the two proposed methods (HPLC and derivative spectrophotometric). The same batch of tablets was also analyzed by an official method [30]. Based on the Student's t-test and the Variance-ratio F-test at 95% confidence level, there is no significant difference between the performances of the three methods as regards accuracy and precision as shown in Table 6.

CONCLUSION

The rapid, simple and fairly reliable proposed methods were employed for the determination of CAR in tablets. The satisfying recoveries and low coefficients of variation confirm the suitability of both proposed methods for the routine analysis of CAR in tablets. While the HPLC method has sensitivity, the derivative spectrophotometric method is more specific than the other methods (2,4). In the proposed HPLC method, the CAR was eluted faster than that in the official method when separated with sharper peak while the derivative spectrophotometric methods have the advantages of lower cost, rapid and environment protecting. The derivative spectrophotometric method serves as an alternative method for the determination of CAR in tablets. Once the optimized conditions were selected, both methods were validated and they showed good performances with respect to linearity, precision and accuracy. Compared to HPLC, the developed derivative spectrophotometry technique was less expensive, low solvent and sample consumption. The results of this study demonstrated that both methods could be used for the routine determinations of CAR in tablets.

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