INTRODUCTION

Carbamazepine (CBZ), 5-H-Dibenz [b.f] azepine-5-carboxamide (Fig.1), is widely prescribed as an anticonvulsant, antiepileptic and antimanic drug. In the body, CBZ is metabolized to an active metabolite, CBZ-10.11 Epoxide (CBZ-EP), which also displays anticonvulsant properties similar to those of the parent compound\(^3,4\). CBZ is poorly soluble in aqueous media and has a high oral bioavailability in humans\(^3\).
Several methods have been reported for the determination of CBZ standards and in pharmaceutical preparations including spectrophotometric methods, spectrofluorimetry methods, gas-liquid chromatography (GC), FT-Raman spectroscopy and liquid chromatography.

Therefore, the purpose of this investigation was to develop and validate a reversed-phase (RP)-HPLC method that could be used for determination of CBZ in pure forms and in pharmaceutical preparations.

**MATERIALS AND METHODS**

**Materials and chemicals**

Carbamazepine (CBZ) was kindly supplied by Novartis (Turkey). HPLC and analytical grade solvent (methanol, acetonitrile) were purchased from Merck (Germany). The water of HPLC was prepared by double glass distillation and filtration through Millipore 0.45 μm. white nylon HNWP 47 mm filters. Three commercial preparations - Tegretol tablet, Karberol tablet and Karbelex tablet, were assayed.

**Chromatographic system and conditions**

A Thermoseparations Spectra HPLC system was applied to perform the analyses. Detection was accomplished using UV 6000 LP photodiode array detector. The Series P 400 gradient auto pump and a Thermoseparations As 3000 autosampler were conditioned at 25°C. Chromatographic software Chromoquest was used for data collection and processing.

Three different analytical columns with various stationary phases were tested: Phenomenex Bondolone RP C18 column (150 x 3.9 mm, 5 μm.), Ace 5 C8 column (150 x 4.6 mm, 5 μm.) and Nucleosil 5 NH2 100A (250 x 4.0 mm, 5 μm).

**Standard solutions**

Standard stock solution of CBZ was prepared with methanol to a concentration 50 μg ml⁻¹ and stored at 4°C. Working solutions (0.25-25 μg ml⁻¹) were prepared by diluting with water appropriate volumes of stock solutions at 10 ml as needed to construct the calibration curves. The working solutions were prepared freshly in analysis daily. The solutions were filtered through a phenex membrane of 0.45 μm pore size (25 mm filter) and transferred to an autosampler vial for analysis. Calibration graphs were prepared using peak area versus concentrations of working solutions in mobile phase. Six replicate 10 μl injections were made for each concentration. The final concentrations of CBZ in the samples were calculated by comparison with peak area obtained with an average of six injections of standard solutions.

**Analysis of pharmaceutical preparations**

Twenty tablets (Tegretol® Karberol® and Karbelex®) were accurately weighed and finely powdered in a mortar. An amount of tablet mass equivalent to content of a tablet (200 mg for Tegretol® and Karberol®, 300 mg for Karbelex®) was transferred to a 50 ml volumetric flask and dispersed in 40 ml methanol. The flask was placed in ultrasonic bath for 15 min. The resulting suspension was diluted to volume with methanol and then filtered. Further dilutions were performed to constitute the calibration graphs for the RP-HPLC method.

**Method validation**

The methods were validated according to the International Conference on Harmonization Guidelines for validation of analytical procedures.
RESULTS AND DISCUSSION

RP-HPLC method
The development of the RP-HPLC method for the determination of drugs has received considerable attention in recent years because of its importance in routine quality control analysis. Different analytical columns with various stationary phases were tested. Good separation from these three columns was achieved using a Phenomenex Bondolone RP C<sub>18</sub> column. The latter was finally used for analysis. A RP-HPLC method was proposed as a suitable method for the estimation of CBZ in pharmaceutical dosage form. The chromatographic conditions were adjusted in order to provide a good performance of the assay. The method involved a mobile phase consisting of acetonitrile-Milli-Q grade water (3:7 v/v) accomplished at 220 nm. The retention time was 8.2 min at a flow-rate of 1 ml min<sup>-1</sup> and the injection volume was 10 µl. The total run time for an assay was approximately 9 min. The integrator attenuation was 8 and the chart speed was 0.2 cm min<sup>-1</sup>. The mobile phase was chosen after several trials with other solvent combinations. Mobile phase selection was based on peak parameters (symmetry, tailing), run time, ease of preparation and cost. Figures 2 and 3 show a typical chromatogram obtained from the analysis of a standard and solution of commercial CBZ using the proposed method. As shown in these figures, CBZ was eluted forming symmetrical peak and well separated from the solvent front. Observed retention time (8.2 min) allowed a rapid determination of the drug. System suitability parameters calculated under the optimized experimental conditions were capacity factor (k') 4.46; symmetry factor 1.05 and column efficiency (n) 4303 plates/m.

Linearity
To determine the linearity of the RP-HPLC method, calibration standard solutions of CBZ were prepared as in the text. The linear ranges were found to be 0.25-25 µg ml<sup>-1</sup> concentration. The regression equation and correlation coefficient (r) obtained by least square regression method were y=449820x+115048 (y: peak area, x: concentration) and 0.9995, respectively. The regression equation calculated from calibration curves given with the standard deviations of slope (S<sub>b</sub>) and intercept (S<sub>a</sub>) on the ordinate are given in Table 1. The linearity of the calibration graph and conformity of RP-HPLC value to Beer’s Law were proven by the high correlation coefficients (r) for the regression equations.

Accuracy and precision
Repeatability is given as inter- and intra-day precision and accuracy evaluated by analyzing three different concentrations of CBZ. Accuracy of the method was checked for six days at three concentration levels at 2.5, 7.5 and 15 µg ml<sup>-1</sup> in six replicates. The results are given in Table 2. The precision of the RP-HPLC method was demonstrated by the relative standard derivation (RSD %) of lower than 5.88% for intra-day and 6.68% for inter-day. Recovery experiments were
performed via standard-addition technique. To fixed and known amount of drug in the pre-analyzed tablet extracts, pure CBZ (standard) was added at three levels and the total amount was found by method. The experiment at each level was repeated six times. The developed RP-HPLC method was applied to three serials of tablets and the percent recoveries obtained are given in Table 3. These results indicated a very good reproducibility of this method.

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**Table 2.** Precision of the methods for determination of CBZ (n=6)

<table>
<thead>
<tr>
<th>Added (µg ml⁻¹)</th>
<th>Found (µg ml⁻¹)</th>
<th>Precision (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(RSD)</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.50 ± 0.0955</td>
<td>3.80</td>
<td>4.40</td>
</tr>
<tr>
<td>7.5</td>
<td>7.45 ± 0.0989</td>
<td>1.29</td>
<td>6.99</td>
</tr>
<tr>
<td>15</td>
<td>14.73 ± 0.196</td>
<td>1.29</td>
<td>1.66</td>
</tr>
</tbody>
</table>

SO D: Standard deviation of six replicate determinations.
RSD: Relative standard deviation.
Accuracy: % relative error (% (tabled-found)/tabled) x 100

**Table 3.** Recovery values of CBZ in pharmaceutical preparations (n=6)

<table>
<thead>
<tr>
<th>Concentration added (µg ml⁻¹)</th>
<th>Mean (RSD%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>98.8 (0.87)</td>
</tr>
<tr>
<td>7.5</td>
<td>99.8 (3.65)</td>
</tr>
<tr>
<td>15</td>
<td>101.5 (2.69)</td>
</tr>
</tbody>
</table>

Mean of six replicate determinations

**Sensitivity**

Limit of detection (LOD) and limit of quantitation (LOQ) were determined by an empirical method that consisted of analyzing a series of standard solutions containing decreased amounts of CBZ. The LOQ is defined as the lowest concentration on the calibration curve at which both accuracy and precision should be within 20%. LOQ value of the RP-HPLC method was determined as 0.07 µg ml⁻¹, and its precision and accuracy were well within the proposed criteria (Table 1). The LOD for CBZ determination was approximately 0.05 µg ml⁻¹.

**Stability**

For the determination of the stability of CBZ in solution at ambient 4°C and -20°C refrigeration temperature, three sets of low (2.5 µg ml⁻¹), medium (7.5 µg ml⁻¹) and high (15 µg ml⁻¹) concentrations were divided into 12 tubes. One set was assayed immediately and taken as standard (100%). One of the sets was stored at 4°C for 24 h and for 1 week. The other set was stored at - 20°C in a deep freezer for 24 h. The remaining set was allowed to stand at ambient temperature for 24 h. The stability measurements were then carried out. The results were evaluated by comparing these measurements with those of standards and expressed as percentage deviation. They are summarized in Table 4.

**CONCLUSION**

This paper describes a reversed-phase HPLC method for determination of CBZ in pure forms and in pharmaceutical preparations with minor sample treatment. The validation studies show good recoveries, precision and accuracy. This method was successfully applied to assay of CBZ in tablets (Tegretol®, Karbex® and Karbelex® (Table 5)). In summary, the reported method can be used for the routine quality control analysis of the investigated drug in pharmaceutical preparations.

**REFERENCES**


