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Simultaneous Determination Of Carbamazepine In Human Plasma By HPLC-DAD: Assay Development, Validation And Application To A Clinical Study

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ABSTRACT

A simple, sensitive, precise, accurate and specific HPLC method for the determination of CBZ in human plasma was developed and validated. The CBZ was isolated from human plasma by liquid-liquid extraction, gave good results with chloroform. The method was linear in the concentration range 0.5 to 25 μg ml^{-1} ($r>0.998$). Recovery for CBZ was greater than 91.7 %. The limit of quantification (LOQ) and the limit of detection (LOD) were 0.15 μg ml^{-1} and 0.10 μg.ml^{-1}, respectively. Inter-batch precision, expressed as the relative standard deviation (RSD), ranged from 0.79 to 6.30 % for intra day and from 1.60 to 1.95 % for inter day and accuracy was better than 90 %. Analysis of CBZ concentrations in plasma samples from six healthy volunteers following oral administration of single dose of CBZ (Tegretol ® CR 200 (200 mg)) provided the following pharmacokinetic data (mean ± SD): C_{max}, 1.550±0.468 μg ml^{-1}; AUC_{0-72} , 52.190±18.942 μg h mL^{-1}; AUC_{0-8}, 68.306±21.698 μg h ml^{-1}; T_{max}, 6,120±6,632 h; $t_{½}$ 34.270±16.787 h; CL, 3.2602 l min^{-1}.

KEYWORDS

Carbamazepine;
Pharmacokinetic;
HPLC.

INTRODUCTION

Carbamazepine (CBZ), 5-H-Dibenz [b,f] azepine-5-carboxomide (Figure 1), is widely prescribed on an anticonvulsant, antiepileptic and antimanic drug[1-2]. In the body, CBZ is metabolized to an active metabolite CBZ-10,11 Epoxide (CBZ-EP) which also displays anticonvulsant properties similar to these

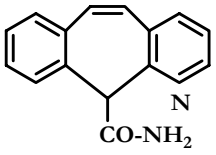

Figure 1: Chemical structure of carbamazepine

of the parent compound[3,4]. The plasma level of CBZ-EP is lower than that of the parent dug, the relative percentage ranging from %10-15[5]. CBZ, poorly soluble in aqueous media, has a high oral bioavailability in humans. The gastrointestinal absorption is characterized as slow, irregular and possibly incomplete[6]. The half-life of 30-35 h occurs after the first dose of CBZ. During multiple dosing, the half-life is decreased to 10-20 h, probably due to autoinduction of the oxidative metabolism of the drug[7]. After single dose of CBZ, elimination follows dose-dependent first order kinetics[6]. Plasma levels of CBZ seem to increase with dose. After the first dose of CBZ, the required time for maximum plasma levels of CBZ may have to be increased until 20-30 h[8].

A number of different methods have been reported for the determination of CBZ in biological fluids, including gas chromatography or high-performance liquid chromatography (HPLC) with UV detection or Enzyme- Multiple Immunoassay[9-38]. However, some of the methods mentioned are poorly reproducible or are expensive or lack selectively.

Therefore, the purpose of this investigation was to develop and validate a method that could be used for the determination of CBZ in real human plasma samples. In the present paper a simple, rapid, sensitive, precise, accurate and specific reversed phase HPLC-DAD assay is described that has been applied to pharmacokinetic studies which required high sensitivity and selectivity.

EXPERIMENTAL

Chemicals and reagents

CBZ was purchased from Novartis (Turkey). Chloroform and methanol were HPLC grade (Merck, Germany). Tribasic sodium phosphate and all other chemicals were analytical grade. Water was Milli-Q grade.

Chromatographic system and conditions

The CBZ analyses were performed on a thermo separations spectra HPLC consisted of UV 6000 LP photodiode array detector, Series P 400 gradient pump and a Thermoseparations As 3000 autosampler. Chromatography was conducted using a mobile phase of acetonitrile- Milli-Q grade water (30:70 v/v) pumped at a flow-rate of 1 ml·min^{-1} through a Phenomenex Bondolone reversed-phase C_{18} column (150 x 3.9 mm, 5 μm, USA). The injection was 10 μL and the peaks was detected at 220 nm. The integrator attenuation was 8 and the chart speed 0.2 cm min^{-1}. Retention time of CBZ was 8.2 min and the total run time for an assay was approximately 9 min.

Preparation of standard solution and controls

Standard stock solution of CBZ (S_o) was prepared with methanol to a concentration 50 μg ml^{-1} and stored at 4°C. 0.5-25 μg.ml^{-1} working solutions (S) were prepared by diluting with water appropriate volumes of stock solutions at 10 ml as needed to construct the calibration curves. Working stock solutions were prepared freshly in every day analysis. Preparation of plasma standard solutions; 0.1 ml of fresh working solution S was used to spike 0.5 ml of blank plasma. The concentrations of the plasma standards (STD) at respective points on the concentration graphs were 0.5, 1, 2, 2.5, 5, 7.5, 10, 15 20 and 25 μg ml^{-1} in human plasma. The quality control (QC) samples at concentration 2.5, 7.5, 15 μg ml^{-1} of CBZ were prepared similarly in human plasma. These samples were used in analysis of plasma samples as quality controls for the purpose of checking recovery of analyte in the daily analyses of plasma samples.

Extraction from plasma

The analytical method of Mac Kichan[24] was used to assay CBZ in plasma. 0.1 ml of calibration standard was added to 0.5 ml plasma sample in a 12 ml capacity glass tube. Following addition of saturated tribasic sodium phosphate solution and 7 mL chloroform were vortexed for 15 min. They were then centrifuged at 1200 g for 10 min. The organic phase was transferred to a clean another glass tube and evaporated to dryness at 40°C under a stream of ni-

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trogen. The residue was dissolved in 1 ml of mobile phase. The solutions were filtered through a phenome nex membrane of 0.45 μm pore size (25 mm filter) and transferred to an autosampler vial for analysis. 10μL volume was injected into the HPLC system for quantitation.

Assay validation

Linearity of the assay was demonstrated over the concentration range 0.5-25 $\mu g.ml^{-1}$ by assaying plasma standards in six replicate at ten separate concentrations on three separate occasions. Peak integration, regression and calculation of concentration were computed using Chromquest Software. The calibration curve was constructed using a weighted (1/x) linear regression of peak-area ratios versus concentrations of analyte in human plasma.

Assay precision and accuracy were determined in conjunction with the linearity studies by assaying on six replicate on three separate occasions using three quality control samples at each of three concentrations (2.5, 7.5, 15 $\mu g.ml^{-1}$). Concentrations of CBZ in quality control samples were determined by application of the appropriate standart curve obtained on that occasion.

Recovery of CBZ was assessed by direct comparison of peak heights from extracted versus non-extracted samples by using six replicate plasma samples at each of ten CBZ concentrations 0.5, 1, 2, 2.5, 5, 7.5, 10, 15 20 and 25 $\mu g.ml^{-1}$. Individual specificity in relation to endogenous plasma components was demonstrated by analysis of a series of randomly selected drug-free samples (n=6).

Pharmacokinetic study

The HPLC method developed was used to investigate the plasma profile of CBZ. Six healthy volunteers (three females and three males) determined by a physical examination and clinical laboratory test results age: 24± 6.6 years, body mass: 59±6.6 kg, height: 165±7.2 cm, participated in the study. Following written informed consent, (the study protocol was approved by the Ethics Committee of the hospital of Ataturk University) each subject received a single 200 mg oral dose of CBZ (Tegretol ® CR 200 commercially available controlled release tablets) with 100 mL of water under fasting conditions. Blood samples (5 mL) were drawn into heparinized test tubes immediately before (0) and at 1, 2, 4, 8, 10, 24, 48, and 72 h. following drug administration. Blood samples were immediately placed on ice after collection and centrifuged at 1500 g for 10 min at 4°C, and the plasma fraction was separated and stored in polypropylene tubes at -20°C until analysis. Before analysis, plasma samples were thawed at room temperature.

RESULTS AND DISCUSSION

Specifity

Typical chromatograms obtained from extracts of a drug-free plasma and a plasma sample obtained from a volunteer 4 h after a single oral dose of Tegretol containing 200 mg CBZ are represented in figure 2. No interfering peaks of plasma components appeared in the chromotograms obtained from extracts of plasma samples. The retention time was 8.2 min for CBZ.

Linearity

The linear regression analysis of CBZ was constructed by plotting the peak area of CBZ (y) versus analyte concentration ($\mu g \cdot ml^{-1}$) in spiked plasma samples (x). The calibration curves were linear for concentrations ranging from 0.5 to 25 $\mu g \cdot ml^{-1}$. A typical calibration curve had the regression equation of $y = 2.415751e^{-0.06}x + 0.70969$ with a correlation coefficient (r) of 0.998. Calibration curves were established on each day of analysis.

Precision and accuracy

Assay precision and accuracy were assessed by assaying three quality control samples in six replicate on three separate occasions. The following validation criteria for accuracy and precision were used to assess the suitability of the method: accuracy should be within 85 to 115 % except at the limit of quantitation where it should be within 80 to 120 %; RSD should not exceed 15 % except at the limit of quantitation where it should not exceed 20 %[29-31]. The precision of the analytic method was determined by repeatability (intra-day) and intermediate precision (inter-day). Assay precision for CBZ was 6.30

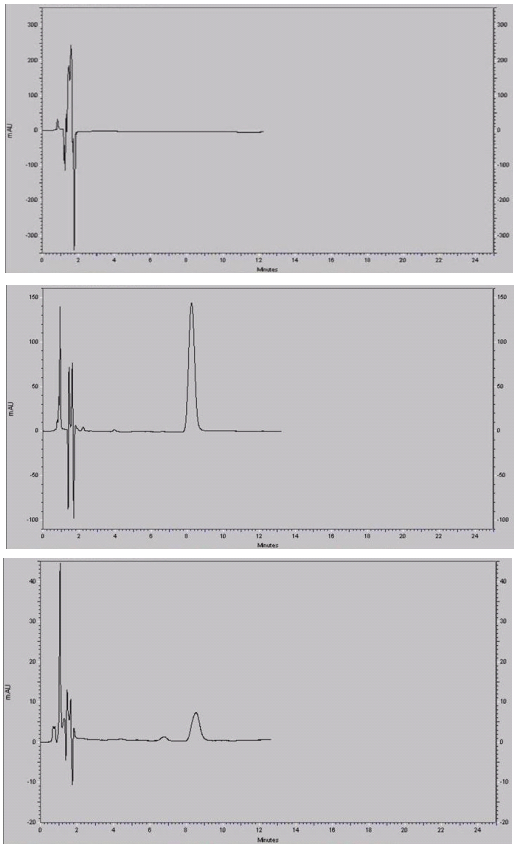

Figure 2: Chromatogram of plasma extracts obtained using the assay procedure. (a) Blank plasma (b) Plasma spiked with 10 μg·ml^{-1} CBZ (c) Plasma obtained from a volunteer 4 h after a single oral dose of Tegretol containing 200 mg CBZ.

% based on RSD values 0.79, 1.41 and 6.30 % for intra day and 1.95 % based on RSD values 1.73, 1.95 and 1.60 % for inter day for samples containing 2.5, 7.5 and 15 μg.ml^{-1}, respectively. Assay accuracy, assessed by calculating the estimated concentrations as a percent of nominal concentrations, was better than 90.0 % (TABLE 1).

Limit of quantification

The limit of quantification (LOQ), defined as the lowest concentration on the calibration curve at which both accuracy and precision should be within %20, was determined to be 0.15 μg·ml^{-1}, the precision and accuracy were good within the proposed criteria (TABLE 1). The limit of detection (LOD) for CBZ defined as a signal-to-noise ratio 3, was 0.10 μg·ml^{-1}.

TABLE 1: Accuracy and precision of the methods for CBZ in quality control samples in human plasma

Nominal concentration (μg. ml^{-1})	Mean Found Concentration (μg. ml^{-1})	Precision[a] (RSD,%)	Accuracy[b] (%)
Intra day			
2.5	2.510±0.020	0.79	100
7.5	6.998±0.093	1.41	93
15	3.412±0.085	6.30	90
Inter day			
2.5	2.495±0.044	1.73	100
7.5	7.108±0.139	1.95	95
15	13.628±0.218	1.60	90

[a] **RSD, Relative standard deviation**
[b] **Accuracy: found concentration expressed in % of the nominal concentration**

Recovery

The recovery of CBZ was determined by direct comparison of absolute peak heights from plasma samples and those found by direct injection of standards of the same concentration prepared in methanol. The mean recoveries for CBZ were 94.2±2.6, 91.7±3.8 and 94±1.8 at the 2.5, 7.5 and 15 μg·ml^{-1} concentrations, respectively (n=6). No effect of the co-extracted biological material was detected.

Aplication to pharmacokinetic study

The present method was used to determine the plasma concentration of CBZ. Figure 3 shows the mean ± standard error of the mean (SEM) plasma concentration-time profile of CBZ. Pharmacokinetic analysis was performed using standard noncompart mental methods[39]. Analysis of CBZ concentrations in plasma samples from 6 healthy volunteers following oral administration of 200 mg of Tegretol ® CR

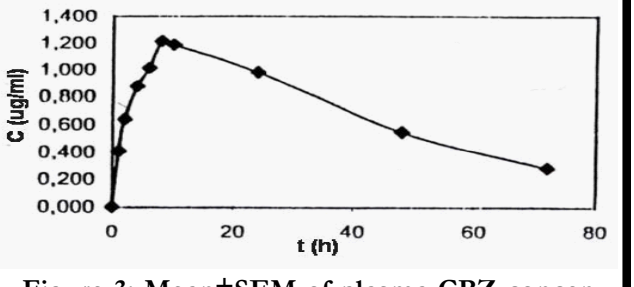

Figure 3: Mean±SEM of plasma CBZ concentration-time curve following a single oral dose of 200 mg Tegretol to six volunteers.

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TABLE 2: Pharmacokinetic parameters for CBZ after a single oral administration of 200 mg Tegretol to 6 volunteers.

Pharmacokinetic Parameter	Mean+SD
C_{max} (μg.mL^{-1})	1.550 ± 0.468
AUC_{0-72} (μg h. mL^{-1})	52.190 ± 18.942
$AUC_{0-?}$ (μg h. mL^{-1})	68.306 ± 21.698
T_{max} (h)	6.120 ± 6.632
$t_{½}$ (h)	34.270 ± 16.787
CL (L.min^{-1})	3.2602

(commercially available controlled release tablets) provided the folloving pharmacokinetic parameters (mean±SD): C_{max}, 1.550 ± 0.468 μg·mL^{-1}; area under curve (AUC), AUC_{0-72}, 52.190 ± 18.942 μg h· ml^{-1}; AUC_{0-8}, 68.306 ± 21.698 μg h· ml^{-1}; T_{max}, 6.120 ± 6.632 h; elimination half-life, $t_{½}$ 34.270 ± 16.787 h; clearance, CL, 3.2602 L.min^{-1} (TABLE 2). The observed values of the pharmacokinetic parameters were comparable to those reported for CBZ in previous studies[28-38].

CONCLUSION

This paper describes a simple, rapid, sensitive, specific, accurate and precise procedure for the determination of CBZ, suitable for the analysis of human plasma samples. The assay was validated to meet the requirements of pharmacokinetic or bioequivale nce studies.

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